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STUDIES ON THE GROWTH PROMOTING FACTOR OF ADULT TISSUE EXTRACTS: I. THE EFFECT OF HEAT; II. THE EFFECT OF DILUTION*

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(Received for publication on August 1, 1943)

In previous communications from this laboratory (Doljanski and Hoffman, 1939; Hoffman and Doljanski, 1939; Hoffman, Tenenbaum, and Doljanski, 1939, 1940; Doljanski, Hoffman, and Tenenbaum, 1942; Doljanski and Hoffman, 1943), it was demonstrated that the ability to strongly activate the growth of cell colonies in vitro is not an exclusive property of extracts of embryonic tissues. Extracts of various adult tissues and organs, too, have been found to stimulate proliferation of cells in vitro to a high degree. It could be shown further that the cell growth promoting activity of tissue extracts is entirely independent of the proliferative state of the tissue from which they are obtained. Extracts of certain mitotically stable tissues, such as adult brain and heart muscle, proved to be more active than the most powerful extracts of proliferating embryonic tissues.

In order to obtain information as to the chemical nature of the growth promoting principles in adult tissue extracts, studies on their general properties have now been undertaken. The present report deals with the effect of (*a*) heat and (*b*) dilution on the cell growth activating power of adult tissue extracts.

Previous records concerning the effect of heat upon the growth promoting activity of tissue extract are limited to the particular case of embryo extract; they are both meagre and contradictory. Carrel (1913) stated that the growth promoting power of embryo extract is reduced when the extract is heated 10 minutes at 56° C., and is removed when extract is heated 10 minutes at 70° C. Hueper, Allen, Russell, Woodward, and Platt (1933) reported their results as follows: "The heating of extract to 70° C. for 15 minutes does not make ex-

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tract unsuitable for the cultivation of cultures which have been grown for some time in a medium containing normal extract. The heated extract is, however, unsuitable for the cultivation of fresh cultures." Lasnitzky (1937) studied the action of heated embryo extract upon the growth of fibroblast cultures in vitro. She found that the growth promoting power of extracts heated for 10 minutes at 70° C. is decreased and that heating for 30 minutes at the same temperature accentuates the effect. The conclusions of this author are based on numerical data.

The effect of dilution on the growth promoting power of embryo extract has been tested by Carrell and Ebeling (1921). These authors found that increasing concentration of embryo extract causes an increase in growth rate of fibroblasts. After the concentration of the juice in the medium had reached 40 per cent no further increase in outgrowth was observed when larger amounts were added.

Willmer and Jacoby (1936) reported that for the low concentrations of embryo extract the cell growth is proportional to the concentration. The growth of "heart fibroblasts" was found to be maximal in 40 per cent extract.

Material and Procedure

1. *Tissue Culture Technique*

The growth stimulating effect of adult tissue extracts was tested on chicken fibroblast colonies growing in Carrel flasks. Flask cultures were prepared as follows: Standardized cover-slip colonies of fibroblast were divided into two equal parts and transferred to flasks: One half was used as a control, the other served for experiment. The solid phase of the medium in which the cultures were planted, was composed of chicken plasma diluted with Tyrode solution in the proportion 1:2. Tissue extracts were added as supernatant fluid phase. The latter was not changed throughout the entire course of the experiment. The course of growth was followed for a period of six days. Growth curves were constructed by planimetric measurements of the surface area of the cultures according to the method of Ebeling.

2. *Preparation of Tissue Extracts*

Extract of cardiac muscle of adult chickens was used in these studies. The hearts were removed from normal adult animals, freed

of fat, and then used in their entirety. The extracts were prepared as follows: the hearts were finely minced with a Latapie apparatus, suspended in four parts of Tyrode solution, then left to stand for 24 hours in the refrigerator. The suspension was centrifuged for five minutes and the supernatant fluid decanted off. The dry matter content of the extracts used in this experiment was 1.6 per cent.

The extracts were heated in a constant temperature water bath. Heating to 60° C. and more causes the formation of a voluminous greyish precipitate, the volume of the precipitate increasing with the temperature and duration of the treatment. The precipitates were removed by centrifugation; supernatant fluid from extracts heated to 60° or 70° C. does not possess the characteristic reddish color of the original extract, but is faintly opalescent and light brown in tone. Extract heated to 100° C. yields after centrifugation a fluid which is almost colorless.

Findings

1. *The Effect of Heat on the Growth Promoting Power of Heart Extract*

It may be seen from Table 1 that the heating of heart extract for 30 minutes at 60° C. is entirely without effect on its growth promoting power. Cell colonies cultivated in medium to which heart extract which had been heated to 60° C. has been added, showed growth rates which are slightly higher than those of controls in unheated extract. Heating for 10 minutes at 70° C. reduced the growth promoting power of the heart extract appreciably; cell colonies grown in an extract heated in such a manner attained approximately one-half the size of controls grown in unheated extract. Prolongation of the heating time to 30 minutes accentuated the effect; cultures fed with tissue which had been heated for 30 minutes at 70° C. attained at an average only one-third the size of controls.

Although heating for 30 minutes at 70° C. considerably reduces the growth promoting power, this treatment does not destroy it completely. As may be seen in Table 2, cell colonies grown in medium to which heart extract which had been heated for 30 minutes at 70° C. is added, attain areas 135 per cent greater than control cultures grown in protective medium (chicken plasma + Tyrode solution). Even heating for five minutes at 100° C. fails to destroy the growth pro-

TABLE 1

THE AREA OF 6-DAY-OLD FIBROBLAST COLONIES GROWING IN HEATED ADULT HEART EXTRACT AS COMPARED TO CONTROLS GROWING IN UNHEATED EXTRACT

Extract heated at 60° C. for 30 minutes			Extract heated at 70° C. for 10 minutes			Extract heated at 70° C. for 30 minutes		
Culture No.	Size of culture in mm.² Contr.	Exp.	Culture No.	Size of culture in mm.² Contr.	Exp.	Culture No.	Size of culture in mm.² Contr.	Exp.
11668/9a	81	86	11585/6a	40	16	11641/2a	100	20
11668/9b	105	105	11585/6b	45	27	11641/2b	95	28
11668/9c	88	88	11585/6c	55	32	11641/2c	98	33
11670/1a	67	100	11619/20a	87	44	11662/3a	95	30
11670/1b	78	78	11619/20b	85	42	11662/3b	84	23
11670/1c	85	110	11619/20c	63	37	11662/3c	92	24
11672/3a	100	107	11637/8a	82	49	11694/5a	65	15
11672/3b	107	107	11637/8b	81	63	11694/5b	43	20
11672/3c	82	82	11637/8c	90	68	11694/5c	74	24
11674/5a	86	98	11639/40a	82	60	11696/7a	89	40
11674/5b	84	84	11639/40b	81	41	11696/7b	85	28
11674/5c	85	105	11639/40c	96	71	11696/7c	84	32
11676/2a	83	85	11658/9a	86	50	11698/9a	90	40
11676/2b	102	100	11658/9b	90	50	11698/9b	85	48
11676/2c	81	81	11658/9c	75	48	11698/9c	100	37
			11660/1a	80	35	11700/1a	105	36
			11660/1b	96	38	11700/1b	75	35
			11660/1c	95	30	11700/1c	104	31
Mean	87.6	94.4	Mean	78.3	44.5	Mean	86.8	30.2
Ratio Exp./Contr.		1.07	Ratio Exp./Contr.		0.57	Ratio Exp./Contr.		0.35

TABLE 2

THE AREA OF 6-DAY-OLD FIBROBLAST COLONIES GROWING IN HEATED ADULT HEART EXTRACT AS COMPARED TO CONTROLS GROWING IN PROTECTIVE MEDIUM (PLASMA + TYRODE SOLUTION)

Extract heated at 70° C. for 30 minutes			Extract heated at 100° C. for 5 minutes		
Culture No.	Size of culture in mm.² Contr.	Exp.	Culture No.	Size of culture in mm.² Contr.	Exp.
11593/42	15	25	11597/8a	8	12
11593/4b	13	18	11597/8b	11	27
11593/4c	17	23	11597/8c	8	17
11595/6a	18	20	11686/7a	16	18
11595/6b	10	23	11686/7b	10	23
11595/6c	10	54	11686/7c	8	19
11611/2a	22	31	11688/9a	9	16
11611/2b	20	28	11688/9b	8	18
11611/2c	6	20	11688/9c	7	17
11613/4a	6	37	11690/1a	8	19
11613/4b	20	35	11690/1b	14	29
11613/4c	16	31	11690/1c	7	20
11615/6a	18	50	11692/3a	16	28
11615/6b	13	38	11692/3b	12	31
11615/6c	13	33			
11633/4a	16	39			
11633/4b	9	50			
11633/4c	10	42			
11635/6a	8	35			
11635/6b	13	29			
Mean	14.1	33.0	Mean	10.1	21.0

moting power of the extracts completely. The final growth area of cultures maintained in medium containing extract which was heated for five minutes at 100° C. is on an average twice as great as the growth area of controls in protective medium, the stimulation varying in degree from culture to culture between 12 and 185 per cent.

2. *The Effect of Dilution on the Growth Promoting Power of Adult Heart Extract*

Adult heart extract diluted with two parts Tyrode solution (33 per cent extract) displayed undiminished growth promoting activity compared with that of undiluted extract. With increasing dilution the growth promoting effect of adult heart extract begins to diminish. Dilution of extract with four parts of Tyrode solution (20 per cent extract) reduces its growth promoting activity to 76 per cent; dilution with nine parts of Tyrode solution (10 per cent extract)—to 44 per cent (Table 3).

TABLE 3

THE AREA OF 6-DAY-OLD FIBROBLAST COLONIES GROWING IN DIFFERENT CONCENTRATIONS OF ADULT HEART EXTRACT AS COMPARED TO CONTROLS GROWN IN UNDILUTED ADULT HEART EXTRACT

10% heart extract			20% heart extract			33% heart extract		
Culture No.	Size of culture in mm.^{2} Contr.	Exp.	Culture No.	Size of culture in mm.^{2} Contr.	Exp.	Culture No.	Size of culture in mm.^{2} Contr.	Exp.
11716/7a	75	30	11706/7a	81	55	11742/3a	75	75
11716/7b	86	32	11706/7b	86	62	11742/3b	81	80
11716/7c	74	30	11706/7c	91	51	11742/3c	90	93
11724/5a	96	38	11708/9a	94	78	11756/7a	60	75
11724/5b	96	42	11708/9b	82	80	11756/7b	90	91
11724/5c	100	32	11708/9c	96	61	11756/7c	108	100
11762/3a	45	30	11710/1a	83	64	11758/9a	84	86
11762/3b	48	41	11710/1b	81	68	11758/9b	91	89
11762/3c	83	41	11710/1c	99	62	11758/9c	75	60
11796/7a	78	31	11722/3a	102	85	11780/1a	67	54
11796/7b	81	30	11722/3b	104	91	11780/1b	90	86
11796/7c	75	41	11722/3c	69	48	11780/1c	75	68
11801a	92	38	11722/3d	89	71	11782/3a	89	96
11801b	92	35	11722/3e	86	60	11782/3b	97	108
11809/10a	84	39	11724/5a	69	65	11782/3c	83	85
11809/10b	82	39	11724/5b	83	65	11784/5a	63	60
11809/10c	96	35	11724/5c	71	53	11784/5b	71	74
Mean	81.3	35.5	Mean	86.2	65.8	Mean	81.7	81.2

TABLE 4

THE AREA OF 6-DAY-OLD FIBROBLAST COLONIES GROWING IN DILUTED ADULT HEART EXTRACT AS COMPARED TO CONTROLS GROWING IN PROTECTIVE MEDIUM (PLASMA + TYRODE SOLUTION)

5% heart extract			1% heart extract		
Culture No.	Size of culture in mm² Contr.	Exp.	Culture No.	Size of culture in mm² Contr.	Exp.
11734/5a	10	22	11774/5a	13	19
11734/5b	14	25	11774/5b	11	18
11734/5c	9	22	11774/5c	13	14
11736/7a	8	26	11776/7a	10	12
11736/7b	11	28	11776/7b	11	18
11736/7c	11	25	11776/7c	12	16
11738/9a	14	28	11778/9a	17	19
11738/9b	11	24	11778/9b	16	19
11738/9c	8	17	11778/9c	15	22
11728/9a	15	28	11786/7a	11	11
11728/9b	11	20	11786/7b	9	10
11728/9c	10	20	11786/7c	8	11
11730/1a	16	26	11788/9a	11	11
11730/1b	16	26	11788/9b	8	11
11730/1c	18	24	11788/9c	9	9
			11790/1a	16	20
			11790/1b	11	16
			11814/5a	18	22
			11814/5b	12	17
			11814/5c	15	20
Mean	12.1	24.1	Mean	12.3	15.7

Table 4 shows that in adult heart extracts of 5 per cent and 1 per cent concentration growth promoting activity is still evident. Cultures in 5 per cent extract attain areas which are in the average twice as great as those of controls in protective medium (chicken plasma + Tyrode solution); the growth activating effect of 1 per cent adult heart extract is very small, but nevertheless detectable.

SUMMARY

1. Heating adult heart extract at 60° C. for 30 minutes had no influence on its growth promoting power.

2. Heating at 70° C. for 10 minutes reduced the growth promoting power of adult heart extract by one-half; heating at 70° C. for 30 minutes—by two-thirds.

3. The growth promoting activity of adult heart extract is not destroyed completely either by heating at 70° C. for 30 minutes or by heating at 100° C. for 5 minutes.

4. Adult heart extract of 1 per cent concentration displayed a very small but detectable growth promoting activity.

5. The growth activating effect of adult heart extract increases proportionally to its concentration and becomes optimal when a concentration of 33 per cent is reached.

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THE INFLUENCE OF SEX HORMONES UPON THE CHEMICAL GROWTH OF THE BRAIN OF WHITE RATS*

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(Received for publication on March 7, 1944)

It had been shown (Weil, '43) that there is a difference in the phospholipid content of the brain of male and female rats, both as to quantity and quality. Furthermore, it was demonstrated that this chemical composition undergoes constant changes during growth, with an abrupt fall in the content of phospholipid during puberty in the brain of the male rat.

The present investigation is concerned with the effect of synthetic sex hormones upon the chemical growth of the brain of the white rat. A priori one might assume that either the chemical composition of the brain is under the direct influence of chromosomal determination or that this chemistry is entirely regulated by the hormones of the glands of internal secretion. In a previous paper (Weil, '41), it was assumed that both factors might play a combined rôle. Such a working hypothesis again suggests itself when one studies the chemical composition of male and female white rats after removal of the gonads before puberty, at which time both qualitative and quantitative composition of corresponding phospholipid fractions are in inverse relationship if compared with the chemical composition of the adult brain (Cf. Tables 2 and 3).

METHODS

Rats were injected with testosterone propionate, 50 micrograms dissolved in .1 cc. of sesam oil per 100 g. body weight being injected subcutaneously every second day; or α-estradiol benzoate, 1 microgram per 100 g. weight dissolved in .1 cc. of sesam oil was given every second day. The experiments may be divided into two groups, rats of our own laboratory stock being used in the first group exclusively

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and Sprague-Dawley rats in the second group. The first group of experiments includes rats in which the gonads had been removed at the early age of 28 to 37 days, and in which the injections were continued until the age of puberty. In the second group rats were included which had been deprived of their gonads shortly before puberty and which were injected for two months thereafter with the hormones of the opposite sex.

The technique which was used for the determination of the different groups of lipids was the same as described in a previous paper (Weil, '43) and was first published by the MacLeans. Its constant use for over 15 years has convinced the author that in combination with the Fisk-Subarrow method of phosphorous determination the values which are obtained for the different lipid fractions are constant enough to allow comparisons between the chemical composition of different groups of rat brains, provided, of course, that one is conscious of the fact that the different fractions obtained are not chemically pure lipids, but probably mixtures of closely related lipids. Furthermore, it is essential to adhere strictly to standards of time of extraction, volume of fluids used, temperature of extraction, etc. The brains of control and experimental animals should be worked up simultaneously, taking care that both groups contain littermates and are raised under the same environmental conditions. Page ('37), who severely criticized the older Fraenkel method of fractionate extraction, used this older Fraenkel method together with the Neumann method of phosphorus determination in his attempt to analyze the "cephalin" fraction of the human brain. The latter method is not very suitable for microdeterminations (Rudy and Page, '30). All analyses of "samples" of human and larger-animal brains are valueless, because they do not take into consideration the varying amounts of gray and white matter, which have different chemical compositions. Only comparisons of whole brains or carefully separated gray and white matter should be accepted as significant and then only if guarantee is given that the rapidly progressing post mortem autolytic changes had been prevented.

Group 1. Injection of Sex Hormones into: A. Immature Male and Female Rats (Table 1). B. Into Castrated and Ovariectomized Rats (Table 2)

It appears from a study of Table 1 that injections of testosterone

TABLE 1

INJECTION OF SEX HORMONES INTO MALE AND FEMALE IMMATURE RATS
(Laboratory Stock. Laboratory Diet. Mean Values.)

No.	Sex Injected	Weight g Original	Weight g Final	Brain *mg*	Hypoph. *mg*	Adren. *mg*	Thyr. *mg*	Alcohol. Extr. *%P*	Cephalin Fraction *%P*
	A. Injection of Homologous Sex Hormones. Duration of Experiment: 32nd to 76th Day.								
10	Male	64	270	1790	7.6	32.8	14.0	2.52	3.07
11	Male + Testoster.	62	301	1800	8.5	29.2	14.4	2.46	3.06
8	Female	58	187	1760	8.8	38.6	10.3	2.59	2.61
10	Female + Estradiol	62	171	1735	11.4	30.2	12.5	2.55	2.63
	B. Injection of Heterologous Sex Hormones. Duration of Experiment: 28th to 76th Day.								
6	Male	38	245	1820	8.6	28.7	15.1	2.49	2.73
8	Male + Estradiol	37	182	1715	10.8	43.7	15.2	2.61	2.58
5	Female	32	161	1660	9.4	36.9	11.8	2.61	2.60
8	Female + Testoster.	34	172	1630	9.1	36.5	14.0	2.71	2.79

into immature male rats and of estradiol into immature female rats has no influence upon sexual maturity. The brains of the injected rats after about six weeks of injections did not differ chemically from the brains of the non-injected rats. Injections of the sex hormones of the opposite sex, however, changed the phospholipid content of the brain accordingly. In studying these figures one should be aware of the fact that before puberty the phosphorus content of the "cephalin" fraction of the male brain is somewhat higher than that of the female brain and that this relation is reversed after puberty. Following injections of estradiol into male rats the *P* content of this fraction is diminished from 2.73 to 2.58 per cent, while injections of testosterone into the normal female rat raised the *P* content of this fraction from 2.60 to 2.79 per cent.

On the other hand removal of the gonads at the early age of 37 days does not change the chemical composition of the male brain, but the brain of the ovariectomized rat undergoes definite changes if studied after maturity (Table 2). The phosphorus of the alcoholic extract is increased from 2.61 to 2.78 per cent, corresponding to a rise of the *P* of the acetone precipitated phospholipids from 2.41 to

TABLE 2

INJECTION OF SEX HORMONES OF THE OPPOSITE SEX INTO CASTRATED AND OVARIECTOMIZED RATS

(Duration of Experiment: 37th to 93rd Day. Laboratory Stock. Sherman Diet. Mean Values.)

No.	Sex	Weight g Original	Weight g Final	Brain mg	Hypoph. mg	Adren. mg	Thyr. mg	Alcohol. Extr. %P	Cephalin Fraction %P
12	Male	71	286	1870	8.2	58.3	15.2	2.45	2.73
10	Castrate	79	231	1785	13.2	62.6	13.4	2.47	2.71
17	Castrate + Estradiol	81	170	1720	12.2	44.1	10.8	2.57	2.60
8	Female	64	187	1710	10.8	44.3	11.5	2.61	2.81
9	Ovariectom.	72	190	1610	8.8	56.8	10.1	2.78	2.73
17	Ovariectom. + Testoster.	74	201	1705	8.0	45.2	12.0	2.53	2.71

2.56 per cent. This again confirms the conclusion of a preceding paper (Weil, '41) that "it appears that the deviation of the ovariectomized animal towards an 'asexual' intermediate level is more pronounced than the deviation of the castrated animal from the normal male." Injections of sex hormones of the opposite sex again change this relationship. The *P* content of the alcoholic extract of the castrate brain injected with estradiol is increased from 2.47 to 2.57 per cent, while that of the spayed rat injected with testosterone is diminished from 2.78 to 2.53 per cent. In Tables 1 and 2 figures giving the weight of the endocrine glands have been included. Injections of estradiol into the immature male increase the weight of hypophysis and adrenal glands; injections of the same hormone into the immature castrate rat reverse the weight of all the three glands towards the female values. On the other hand injections of the male sex hormone into spayed rats changed these values towards the male endocrine formula.

Group 2. Injections of Sex Hormones of the Opposite Sex into Rats which had been Castrated and Ovariectomized at about the Age of Puberty

The changes of the endocrine glands in this group are still more pronounced than in the immature animals. Most impressive is the influence of estradiol injections into castrates. Body weight and length together with the heart weight are considerably reduced and the weight of all three endocrine glands changes to the female formula.

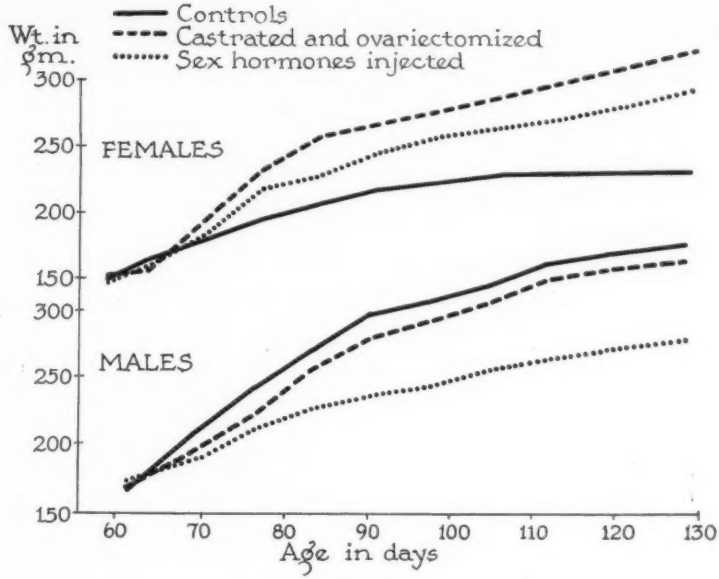

FIGURE 1

COMPARISON OF THE WEIGHT OF WHITE RATS AT DIFFERENT AGES UNDER THE INFLUENCE OF CASTRATION, OVARIECTOMY, AND INJECTION OF HETEROLOGOUS SEX HORMONES INJECTED AFTER REMOVAL OF THE GONADS

(Sprague-Dawley Rats were Operated at the Age of 60 Days. The Castrated Rats were Injected with α-Estradiol Benzoate and the Ovariectomized Rats with Testosterone Propionate. Cf. Text.)

The plump, adipose castrate changes into a smaller, more slender rat with smooth hair which is in contrast to the rough, rugged appearance of the male hair. The chemical composition of the brain changes accordingly. It is best expressed in the *P* values of the "cephalin" fraction. In the castrate injected with estradiol it rises from 2.27 to 2.45 per cent, the female value, and in the spayed rat injected with testosterone it is diminished from 2.30 to 2.21 per cent.

DISCUSSION

The unique effect of the sex hormones reversing the chemistry of the brain differentiates them from the effect of the other endocrine glands. Hyper- and hypo-thyroidism changes the quantity of the phospholipids but not their quality (Weil, '37 and '41). Removal

TABLE 3A

INJECTION OF SEX HORMONES OF THE OPPOSITE SEX INTO CASTRATED AND OVARIECTOMIZED RATS

(Duration of Experiment 60th to 129th Day. Sprague-Dawley Rats. Laboratory Diet. Mean Values.)

No.	Sex	Weight *g* Orig.	Weight *g* Final	Length *mm* Body	Length *mm* Tail	Brain *mg*	Kidn. *mg*	Heart *mg*	Hypoph. *mg*	Adren. *mg*	Thyr. *mg*
5	Male	169	352	247	185	1880	2450	995	9.8	47.3	19.7
6	Castrate	170	348	238	208	1895	1995	935	14.5	44.7	16.9
10	Castrate + Estradiol	169	275	226	192	1885	2070	815	19.5	65.2	15.4
5	Female	149	223	217	170	1795	1710	710	12.6	60.7	13.0
6	Ovariectom.	151	324	230	192	1860	1935	860	12.4	44.0	17.5
10	Ovariectom. + Testoster.	145	294	229	194	1805	1830	8.	11.4	52.0	17.4

TABLE 3B

Sex	Acetone Extract % of Dry	Alcohol Extract % of Dry	Alcohol Extract %P	Precipitate % of Alc. Extract	Precipitate %P	Cephalin Fraction % of Precipitate	Cephalin Fraction %P
Male	15.6	31.8	2.21	16.6	2.00	45.1	2.28
Castrate	18.3	28.4	2.33	15.1	2.06	40.9	2.27
Castrate + Estradiol	15.9	30.7	2.23	19.4	2.11	43.0	2.45
Female	14.2	32.2	2.35	21.0	2.04	53.4	2.43
Ovariectom.	14.2	32.6	2.13	20.2	1.97	44.0	2.30
Ovariect. + Testoster.	14.1	32.9	2.22	22.6	1.80	46.4	2.21

of the adrenal glands will not reverse the chemical constitution, but emphasizes the "male" or "female" chemical make-up of the rat brain (Weil and Groat, to be published). Removal of the hypophysis will tend to increase the phosphorous content of the different lipid fractions, but in both male and female brains alike (Weil and Hetherington, to be published). The question arises whether this effect of the sex hormones consists in altering the chemical constitution of the different phospholipids, or whether only the proportion in the mixture of essentially the same phospholipids has been changed. In the instance of the different proportions of lysine and arginine in male and female brains of man and monkeys (Block, '37) it appears that such differences are only of a quantitative nature. However, the extreme

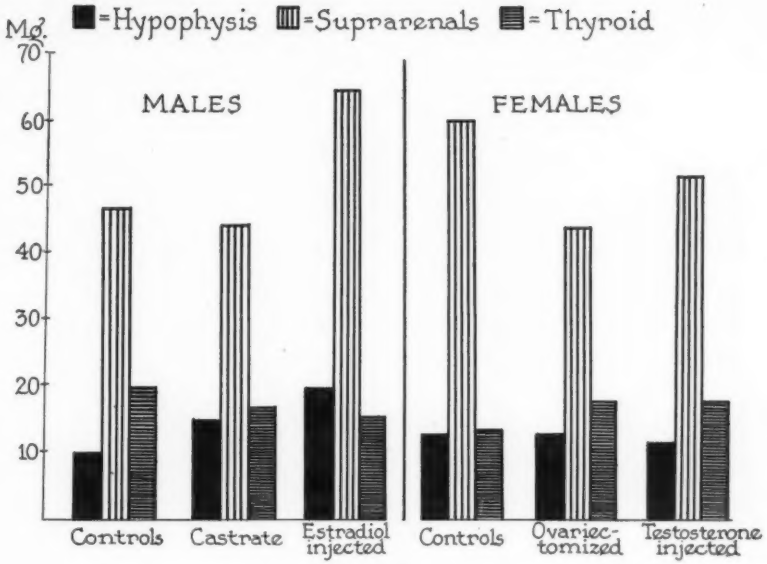

FIGURE 2

THE EFFECT OF CASTRATION, OVARIECTOMY, AND INJECTION OF HETEROLOGOUS SEX HORMONES UPON THE GROWTH OF GLANDS OF INTERNAL SECRETION

(Rats of Group 2. Cf. Text.)

difficulty of qualitative and quantitative analysis of the phospholipids makes a definite answer to this question impossible at the present stage of this investigation. One has to be satisfied with the statement that there exist definite differences in the chemical composition of the male and female brain of man, monkeys, and white rats. The question whether those differences are of a qualitative or quantitative nature can only be answered after more progress in our analytical methods has been made.

SUMMARY

1. Injections of homologous sex hormones into immature male and female white rats will not change the chemical composition of their brains.

2. Injections of the sex hormones of the opposite sex will change

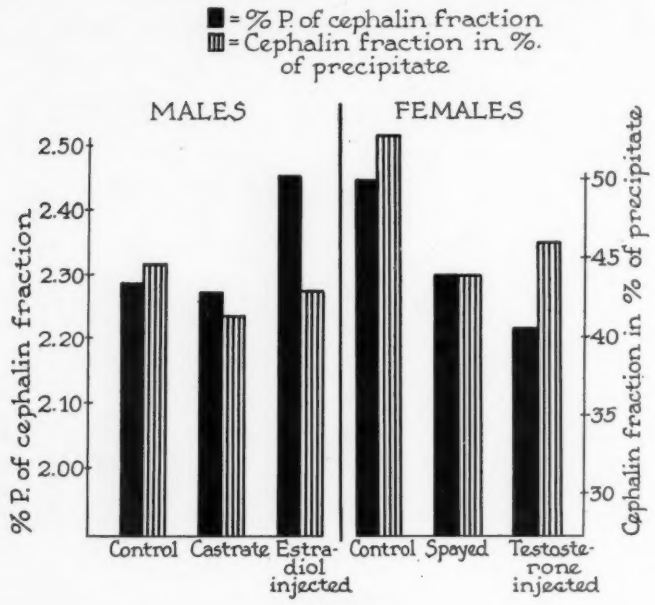

FIGURE 3

THIS FIGURE DEMONSTRATES THE INFLUENCE OF INJECTION OF HETEROLOGOUS SEX HORMONES UPON THE "CEPHALIN" FRACTION OF THE BRAIN OF CASTRATED AND OVARIECTOMIZED RATS FOLLOWING INJECTION OF HETEROLOGOUS SEX HORMONES (Cf. Text.)

the immature male brain into a chemically female brain and vice versa. Such reversals are demonstrated by the analysis of different phospholipid fractions.

3. The reversal of the brain chemistry can also be demonstrated in the brain of rats after the removal of their gonads and injection of the sex hormones of the opposite sex.

4. This reversal can be demonstrated regardless of the age at the time of the removal of the gonads, before or after puberty.

5. The endocrine formula may be changed to that of the opposite sex by injection of heterologous sex hormones, both in normal rats and in rats deprived of their gonads.

Testosterone propionate and α-estradiol benzoate were kindly supplied by Ciba Pharmaceutical Products, Inc.

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TOLERANCE OF LARVAE OF DROSOPHILA FOR NUCLEIC ACID COMPONENTS: THYMINE*

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In this series of investigations, a study is being made of the tolerance of Drosophila larvae for the pure derivatives of the nucleic acids. The tolerance levels have been determined for the purine and pyrimidine bases of ribonucleic acid (Wilson, 1942, 1943a, 1943b, 1943c). The present investigation deals with the tolerance of incompletely fed larvae of *Drosophila melanogaster* for the pyrimidine, thymine, which with cytosine makes up the pyrimidine nucleotides of desoxyribonucleic acid.

The nucleus of cells contains ribonucleic acid in the nucleolus and desoxyribonucleic acid in the chromatin, while ribonucleic acid has been identified in the cytoplasm. Yeast, the natural source of protein for Drosophila, contains both types of nucleic acid but the ribose type is probably the predominant form. It is generally thought that for most organisms, purine, pyrimidine, and the carbohydrate units of nucleic acids are not essential dietary constituents. Cellular nucleoproteins may be completely synthesized from endogenous organic substances (Barnes and Schoenheimer, 1943). Addition of pure components to the diet may, however, so modify growth and development that some clue as to the specific rôle of these derivatives in the growth processes may be obtained. In this series of experiments it was hoped that, in addition to the determination of the concentration of thymine which would be tolerated without apparent modification of normal growh and development, some specific rôle in growth processes might be noted in Drosophila.

The organization of experiments is similar to that used in previous investigations (Wilson, 1941, 1942). Sterile eggs of known age are added to a sterile medium containing one-third the normal require-

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TABLE 1

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH THYMINE. AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

Age	Instar	Control			M/50			M/100			M/200		
		No.	%	Length	No.	%	Length	No.	%	Length	No.	%	Length
48	1	65	42	1.53	99	100	1.42	70	70	1.51	33	38	1.54
	2	89	58	2.09				29	30	1.87	52	62	1.97
76	1				4	5	1.23						
	2	88	54	3.01	71	95	2.46	69	90	2.87	33	41	2.93
	3	76	46	3.73				8	10	3.64	47	59	3.59
96	2				53	72	2.88	6	6	2.44			
	3	123	100	5.00	21	28	3.04	86	94	4.23	65	100	4.76
120	2				9	10	2.83						
	3	90	78	5.04	78	90	4.29	57	100	5.06	14	78	4.92
	P	26	22								4	22	
144	3	1	1		62	100					1	2	
	P	49	99					61	100		48	98	
12 da.	P				62	100		7	17				
	I	46	100					36	83		31	100	
14 da.	P				55	90							
	I				7	10		39	100				

P—pupae.
I—imagoes.

ment of yeast plus sucrose, salts, and vitamins. The larvae are allowed to grow until the first ecdysis between first and second stadia is in progress at approximately 48 hours after egg laying. The larvae are then killed by the hot water method, the length measured and their instar recorded. The same procedure is carried out at the time of the moult between second and third stadia at about 76 hours after egg laying. Other records are made at 96 and 120 hours after egg laying. Rate of pupation and time of eclosion are recorded as percentages of the total number of animals observed.

Swedish-b wild type *Drosophila melanogaster* were used in all the experiments. The thymine was prepared and purified by Dr. Theodore Lavine of the Research Institute of the Lankenau Hospital, Philadelphia.

The following concentrations of thymine were tested, M/50, M/100, M/200, M/400, M/500, M/1,000, M/5,000, M/10,000. Three experiments using test and control animals were performed for each concentration.

Discussion and Results

Table 1 shows the results of treatment with the higher concentrations of thymine. Data include the length in millimeters and the percentages of animals in each instar at 48, 76, 96, and 120 hours ± 1 hour after oviposition. The rate of pupation and emergence of adults is shown by percentages. When the average lengths of the experimental larvae are plotted against those of the control larvae (Figure 1), it is evident that there is a definite retardation of growth in length at concentrations of M/50, M/100 and M/200 thymine. This effect is most pronounced at a concentration of M/50 in which the larvae fail to pupate until the 9th day and the imagoes are four or five days later than control animals in emerging. At a concentration of M/100 growth in length is slightly retarded and emergence of imagoes postponed only 24 hours. No significant difference in time of emergence was noted in animals treated with M/200 thymine although there was a slower rate of growth in length. Concentrations of M/400 and lower do not retard development and are not effective in modifying the growth in length.

Hammett and Lavine (1937) found that, in *Obelia,* thymine enhanced proliferation and differentiation. There are few other reports

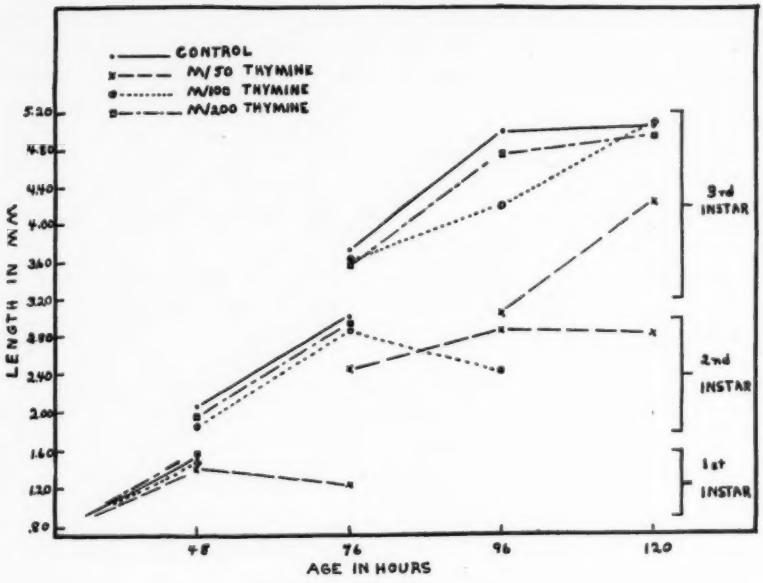

FIGURE 1

RATE OF GROWTH OF LARVAE TREATED WITH THYMINE

of the effect of thymine on growth, but investigators of the specific effects of the pyrimidines confirm the observations of Hammett and Lavine concerning proliferation (Knight, 1937, for bacteria, Lwoff and Dusi, 1938, for Protozoa). It has also been suggested that the substances produced by X-rays which accelerate wound healing (growth by proliferation and differentiation) are probably pyrimidines and purines (G. B. C., 1941).

It is difficult to test proliferation in Drosophila larvae since growth is mainly by increase of cell size. This latter process was not modified for, at no concentration, was there an increase of rate of growth in length. Differentiation as measured by the rate of ecdysis at 48 hours is, however, accelerated in animals treated with concentrations from M/1,000 to M/400 (Table 2). There is no significant difference in the moulting rate of animals treated with concentrations lower than M/1,000. Evidence for acceleration of differentiation is based on

TABLE 2

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH THYMINE AGE IN HOURS FROM MEDIAN HOUR OF OVIPOSITION

Age	Instar	Control			M/400			M/500			M/1,000		
		No.	%	Length	No.	%	Length	No.	%	Length	No.	%	Length
48	1	65	42	1.59	20	18	1.44	36	31	1.52	44	40	1.52
	2	89	58	2.04	92	82	1.95	80	69	2.03	66	60	2.19
76	2	88	54	2.93	90	56	2.95	56	57	2.95	55	54	3.04
	3	76	46	3.72	70	44	3.80	42	43	3.69	46	46	3.90
96	3	123	100	4.96	120	100	5.05	60	100	4.96	38	100	4.76
120	3	90	78	5.04	21	84	5.04	48	94	5.00	32	89	4.90
	P	26	22		3	16		3	6		4	11	
144	P		99			100			100			100	
12 da.	I		100			100			100			100	
Age		Control			M/2,000			M/5,000			M/10,000		
48	1	12	17	1.55	12	16	1.48	2	3	1.57	6	12	1.43
	2	57	83	2.29	61	84	2.16	69	97	2.32	43	88	2.34
76	2	24	34	3.07	19	35	2.92	23	32	2.65	17	33	2.85
	3	48	66	4.00	35	65	4.30	50	68	4.01	35	67	4.30
96	3	82	100	4.88	90	100	5.00	102	100	4.96	86	100	4.89
120	3	16	70	5.15	21	60	5.19	22	69	5.07	40	80	5.14
	P	7	30		14	40		10	31		10	20	
144	P		98			100			100			100	
11 da.	I		100			100			100			100	

P—pupae.
I—imagoes.

the percentages of individuals which have reached the second instar at 48 hours. In the case of those treated with M/400, M/500, and M/1,000 thymine the data show that in every experiment a greater number have moulted in the tests than in the control animals. The effect does not persist into the second moult; so that thymine seems to accelerate differentiation early in larval history. It might be noted in this connection that the retardation in the higher concentrations is also more pronounced earlier rather than late in larval life. Thymine may then exert its effect during the first few days of development rather than throughout larval and pupal life.

Those flies which emerged from the cultures treated with M/50, M/100, and M/200 thymine, though retarded in development, were able to produce fertile eggs and offspring capable of normal development. A large proportion of those flies treated with M/50 thymine during their larval life showed aberrations of various kinds. No significant increase of abnormal features appeared at any other concentration. One of the most persistent anomolies was a darkening of the chitin, in some cases similar to the mutant, ebony. Various eye and wing deformities were also observed. It has been reported by Gershenson (1939) that feeding of thymonucleic acid in large quantities causes a high mutation rate. These observations were not confirmed by Muller (1941) who did however observe a certain number of anomolies among the experimental animals. Further experiments will be necessary to determine whether the effect of thymine is similar to that of thymonucleic acid or not.

In animals treated with M/50 thymine and to a lesser extent in those treated with M/100 thymine there was a distinct difference in the appearance of the Mapighian tubules of the adults when compared with those of control animals. In the test imagoes there was a decided decrease in the number of the yellow pigment particles of the cells making up the walls of the tubules. According to Brehme and Demerec (1942) there is a range, in different Drosophila mutants, from colorless droplets through pale yellow to bright yellow droplets in the cells of the tubules. This pigment as shown by Beadle (1937) is associated with eye color mutants and is modified by dietary constituents (Khouvine, Ephrussi, and Chevais, 1938). Little is known of its fundamental physiological implications or functions. The fact that body pigmentation and eye conditions were modified by this high

concentration of thymine may also indicate disturbance of the pigment metabolism. Certain natural pigments do contain pyrimidines in their molecules (Chrometzka, 1937) but much more investigation is necessary before the problem can even be clearly stated. Perhaps thymine does play a specific rôle in the pigment formation of certain structures in Drosophila.

Summary

1. Larvae of Drosophila tolerate thymine in concentrations below M/2,000 without evidence of disturbance of growth in length or emergence of imagoes.

2. Concentrations of M/1,000 to M/400 thymine slightly accelerate differentiation during the first instar.

3. Growth in length and formation of puparia by larvae of *Drosophila melanogaster* are definitely retarded by addition of M/200 or higher concentrations of thymine.

4. There is evidence that thymine is involved in the metabolism of pigments in *Drosophila melanogaster*.

I am particularly indebted to Dr. Frederick S. Hammett for advice and criticism and to Dr. Jack Schultz for suggestions concerning certain genetical aspects.

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TOLERANCE OF LARVAE OF DROSOPHILA FOR AMINO ACIDS: ARGININE

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Preliminary to an analysis of the rôle played by a given substance in developmental growth is the discovery of a mixture of chemical compounds in proportions consistent with normal growth. For Drosophila, fat, vitamin, salt and carbohydrate requirements for a synthetic diet have been fairly well established; but the concentrations of amino acids and nucleic acids have not been so well determined nor has their specific effect been investigated to any extent. Hammett (1942) emphasized the fundamental importance of each derivative of protein in the investigation of developmental growth. The nature and concentration of these derivatives is therefore of importance in the study of growth in Drosophila. It is the purpose of this series of investigations to determine the concentrations of amino acids which will be tolerated by the developing fly and at the same time to observe various indices of growth changes in order to obtain clues to specific effects of the amino acid in question. The present paper concerns the tolerance in *Drosophila melanogaster* for the amino acid, arginine. Observations of any modifications of processes concerned in developmental growth are also recorded and considered in terms of the effect produced by various concentrations of arginine.

Some investigations of the amino acid requirements of Drosophila have been made. In 1935 Van't Hoog found that with casein as a source of protein, normal pupation of the fruit fly would occur only if the amino acids, cystine and tryptophane, were added. Lafon (1939) used casein, muscle, or fibrin to supply protein and concluded that tryptophane, cystine, arginine, and histidine were essential for growth. Tatum (1941) successfully determined the vitamin requirements of Drosophila by using hydrolyzed casein supplemented with tryptophane to meet the protein needs.

Lafon (1939) tried a mixture of 18 amino acids but was unsuccess-

ful in obtaining growth. He did not supply the required vitamins so it is impossible to evaluate his experiments. As pointed out by Kline (1943), animals fed such a mixture of amino acids often die because of an avitaminosis rather than because of the lack of any necessary amino acid. Attention is also called to the fact that at certain concentrations pure amino acids may be toxic.

There are several methods of determining the significance of any amino acid to growth. Among these, diets of incomplete proteins may be used; such a method was used by Van't Hoog and Tatum. Secondly, diets may be devised from which amino acids have been extracted by chemical methods; Lafon used this procedure. In the third method amino acids are used to supplement a diet inadequate in quantity but not in quality. This latter method is used in our investigations. It is possible to retard but not modify the essential pattern of growth in Drosophila by feeding a basal diet inadequate in quantity. Theoretically, addition of proper amino acids and perhaps nucleic acids in the correct proportions should result in resumption of the normal rate of growth in animals so fed. This method is fruitful in obtaining preliminary information and although seldom successful in solving problems conclusively, it is useful and data so obtained may be used to devise a completely synthetic medium.

A great number of confusing reports concerning arginine and its effect on growth have been published. Although the primary concern of students of developmental growth is not an investigation of the so called essential protein constituents of the diet, results of such studies do however contribute pertinent facts. The value of arginine in the diet varies; for some species it does not modify growth, for others it is essential, for still others it accelerated growth. Rose (1938) included arginine with the amino acids essential for the rat. It may be synthesized but not in amounts sufficient to maintain normal increase in weight. More recent experiments indicate that for the rat it is particularly involved in the synthesis of plasma protein (Malden, Carter, Kattus, Miller, and Whipple, 1943). Arginine is essential for increase of weight and production of feathers in the chicken (Hegsted, Briggs, Elvehjem, and Hart, 1941). It is also essential for the growth by cell division of the ciliate *Colpidium striatum* (Kline, 1943). Arginine is essential for normal pupation in Drosophila (Lafon, 1939).

Arginine may in some cases increase the rate of growth. Gilroy (1930) showed that it accelerated the growth of tumors in the mouse. He suggested that there was increase in nuclear substance which led to an increase in cell division. An indication that such might be the case is shown in the observation of Stedman and Stedman (1943) that chromosomin, a protein from the nucleus of cells contains a high percentage (7 per cent) of arginine. The presence of arginine increases the rate of multiplication of cells in yeast (Mitchell and Roger, 1940). Hoffman and Gudernatsch (1931) demonstrated that tadpole growth is stimulated by arginine but that differentiation is not furthered. Oxygen consumption in *Escheridchia coli* is accelerated by arginine (Tang and Houch, 1943). In *Lactobacillus arabinosus*, arginine stimulates cell division at two concentrations but has an inhibitory effect at an intermediate concentration (Shankman, 1943). The guanidine group from arginine is directly utilized for creatine formation in man, rat, and the dog. This creatine may be stored in the muscles for some time and thus increase their total mass (Beard, 1941).

Hammett and Chatalbush (1935) showed that arginine in concentrations greater than M/50,000 seemed to retard developmental activity of *Obelia* hydranths. Miller and Reimann (1943) observed that for rabbit ova, l-arginine also retarded differentiation thus producing smaller ova than the control. They have evidence that it stimulates blastocyst formation but retards cytological differentiation. These last two experiments corrorborate the effect reported by Hoffman and Gudernatsch (1931) for the tadpole.

In dogs, no modification of growth occurred when they were fed on a diet entirely lacking in arginine (Rose and Rica, 1939). Contrary to Geiling (1917), Baur and Berg (1943) found that arginine did not affect growth rates of mice and that they can probably synthesize it fairly rapidly.

Arginine is particularly concerned with invertebrate muscle physiology since it is the precursor of the phosphoprotein which presumably plays the same rôle in invertebrate anaerobic muscle contraction as phosphocreatin does in vertebrate muscle function. Evidence was obtained from phosphorylation of arginine by crab muscle (Lehman and Pollack, 1942).

In view of these varied reports on the functions and effects of

arginine, it seems of interest to investigate the requirements of Drosophila larvae for arginine, to determine their tolerance for it and to shed some light on its specific rôle in growth.

The work was done at The Marine Laboratory of the Lankenau Hospital Research Institute under the direction of Dr. Frederick S. Hammett, whose suggestions and advice contributed much to the development of the problem.

Materials and Methods

A pure inbred stock of wild type *Drosophila melanogaster* Swedish-b was used for this investigation. The culture bottles were kept in the dark at 25 ± 1° C. For preliminary experiments, banana agar was used for the stock medium because it stimulated the laying of a great number of eggs. Because of scarcity of bananas and agar, however the later experiments of the series were carried out on animals reared on an agarless medium devised by Lewis (1942) as modified by Schultz (1943).

Flies, two to three days old, were removed from the stock bottles and placed in wide mouthed bottles which contained 15 cc. of the stock medium and fresh live yeast. Fifteen to 20 pairs of flies were placed in each bottle and 20 to 40 such bottles were prepared for each experiment. These flies were fattened for three days by daily feeding of live yeast suspension and then transferred to 125 cc. egg collecting bottles, into which had been inserted a pyralin spoon covered with a thin layer of agar for ebb collection (Palmer, 1939). At the end of two hours the spoons were removed and the eggs sterilized in 85 per cent alcohol for 45 minutes. Large numbers of sterile eggs were transferred with a bulb pipette to agar plates, plain agar for the control eggs and agar to which a definite concentration of arginine had been added for the experimental eggs. The plates were examined at 17 hours after the mean hour of egg laying and all larvae removed and discarded.

After the 17th hour it was often possible to pick out the larvae as soon as they hatched so that the age was known accurately. For most of the experiments the 600 or more larvae required for each experiment were transferred within a two-hour period so that the age was known ±1 hour from hatching time.

The culture media was a modification of one devised by Schader (1939). The constituents of the salt mixtures are as follows:

Solution A

8 gm. potassium sodium tartrate
2 gm. ammonium sulphate
5 gm. magnesium sulphate
250 cc. distilled water

Solution B

5.00 gm. tartaric acid
0.65 gm. potassium dihydrogen phosphate
250.00 cc. distilled water

Solution C

0.25 gm. calcium chloride
250.00 cc. distilled water

These are prepared and sterilized and may be kept in the refrigerator for use in several experiments. A supplementary vitamin solution (Tatum 1941) was made up from pure U.S.P. vitamins as follows:

50 mg. thiamin
125 mg. riboflavin
250 mg. nicotinic acid
250 mg. calcium pantothenate
250 cc. distilled water

The following culture medium was then sterilized as indicated and mixed together:

Mixed and sterilized

3 gm. agar
2 gm. sucrose
3 or 5 or 10 gm. Fleishman's yeast
50 cc. distilled water
50 cc. solution *A*
50 cc. solution *C*

Sterilized separately

50 cc. solution *B*

Not sterilized

0.2 cc. vitamin solution

For the tests appropriate concentrations of arginine were added to 100 cc. of the above mixture.

Twenty-four experiments were performed in which the following concentrations of arginine were tested: M/100, M/200, M/400, M/800, M/2,000, and M/10,000. In order to be sure that arginine

was not already in excess of the requirements, three different concentrations of yeast were used. In the experiment using 10 grams of yeast per 100 cc. of medium pupation occurred shortly after 96 hours from hatching; using 5 grams of yeast pupation occurred at 100 hours after hatching; and with 3 grams of yeast per 100 cc. of culture medium, pupation occurred at 116 hours after emergence of larvae from the egg.

Twelve test and 12 control 5 cm. Petrie dishes and in addition six test and six control 4 in. test tubes were prepared for each experiment. The test tubes are more satisfactory for observation of late larval and pupal stages while the Petrie dishes facilitate removal of larvae. Three cc. of warm sterile medium were pipetted into each of the dishes and tubes. Tests and controls were run simultaneously from eggs obtained at a single egg laying.

Throughout the experiment all precautions to insure sterility were taken. The glassware was sterilized by boiling for one hour on two successive days in distilled water. It was then dried in the oven over night at 150° C. Any culture showing contamination was discarded.

Newly emerged larvae were transferred in lots of 20-25 directly from the sterile agar plates to the medium to be tested. Both tests and controls were placed in large sterile crystallizing dishes which were kept in an incubator at 25 ± 1° C. Larvae were killed by the boiling water method (Alpatov, 1929) at four intervals, the duration of which depended upon the concentration of yeast in the culture medium.

Measurements of length and width were made with an ocular micrometer in a 10-X eyepiece used with a 48 mm. objective. Length was measured as described by Alpatov, and width from a dorsal view at the posterior border of the seventh abdominal segment (Brehme, 1939).

Results and Discussion

Tables 1, 2, and 3 show the effect of treating larvae with M/100, M/200, M/400, and M/800 arginine. Figures 1, 3, and 5 show graphically the effect of arginine on length while Figures 2, 4 and 6 show the effect on width. No evidence of toxicity or retardation was shown at any of these concentrations. A concentra-

TABLE 1

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ARGININE (FIVE GR. YEAST PER 100 CC.)

Age	I	Control No.	Control Length	Control Width	M/800 No.	M/800 Length	M/800 Width	M/100 No.	M/100 Length	M/100 Width
30	1	22	1.52	.273	24	1.53	.256	20	1.52	.270
	2	22	1.90	.300	23	1.91	.300	23	1.87	.287
65	2	12	3.00	.437	18	2.97	.442	9	2.99	.442
	3	9	3.58	.483	21	3.63	.500	8	3.65	.497
77	2	0			2	3.09	.450	0		
	3	21	4.12	.512	22	4.18	.549	20	4.52	.615
100	3	23	5.17	.740	38	5.29	.740	46	5.34	.753

I—instar.

TABLE 2

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ARGININE (FIVE GR. YEAST PER 100 CC.)

Age	I	Control No.	Control Length	Control Width	M/800 No.	M/800 Length	M/800 Width	M/200 No.	M/200 Length	M/200 Width
30	1	22	1.49	.259	13	1.49	.254	15	1.52	.250
	2	54	1.91	.293	50	1.95	.290	48	1.96	.291
65	2	21	2.93	.400	16	2.99	.394	16	2.94	.384
	3	40	3.65	.484	58	3.94	.499	54	3.96	.521
77	2	2	2.93	.371	1	3.00	.375	3	3.08	.375
	3	63	4.15	.513	63	4.39	.556	52	4.45	.570
100	3	58	4.88	.614	73	4.93	.658	45	4.99	.682

I—instar.

TABLE 3

AVERAGE LENGTH AND AVERAGE WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ARGININE (FOUR EXPERIMENTS USING FIVE GRAMS OF YEAST PER 100 CC.)

Age	I	Control			M/100			M/200			M/400			M/800		
		No.	Length	Width	No.	Length	Width	No.	Length	Width	No.	Length	Width	No.	Length	Width
30	1	131	1.48	.245	53	1.52	.245	42	1.53	.246	33	1.52	.252	45	1.51	.245
	2	176	1.76	.274	140	1.88	.288	87	1.96	.291	97	1.94	.291	51	1.90	.293
65	2	78	2.89	.409	21	2.99	.439	30	2.97	.403	23	2.91	.395	18	2.97	.442
	3	86	3.61	.477	32	3.52	.488	92	3.92	.508	88	3.99	.524	21	3.63	.500
77	2	13	2.93	.421	2	2.96	.412	3	3.18	.375	2	3.00	.375	2	3.19	.450
	3	189	3.97	.501	50	4.33	.572	65	4.31	.556	72	4.32	.552	22	4.18	.549
100	3	256	4.81	.625	153	5.12	.695	119	5.11	.701	150	4.99	.668	82	5.17	.713

I—instar.

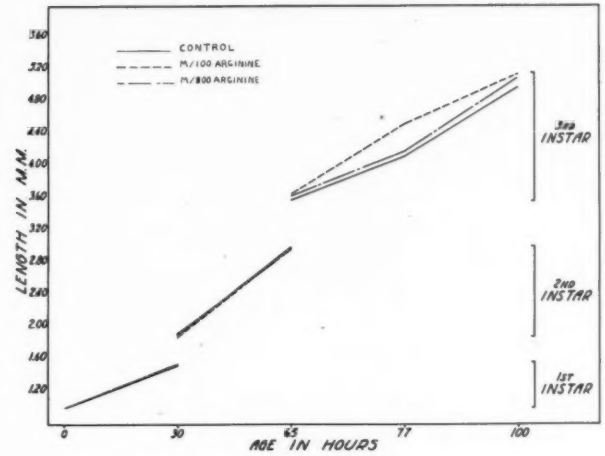

FIGURE 1

GROWTH IN LENGTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

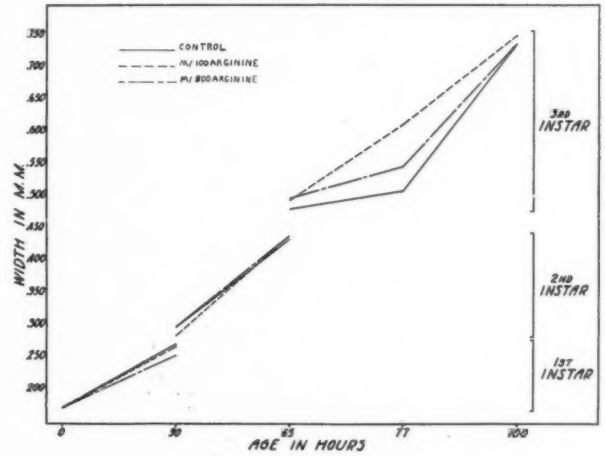

FIGURE 2

GROWTH IN WIDTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

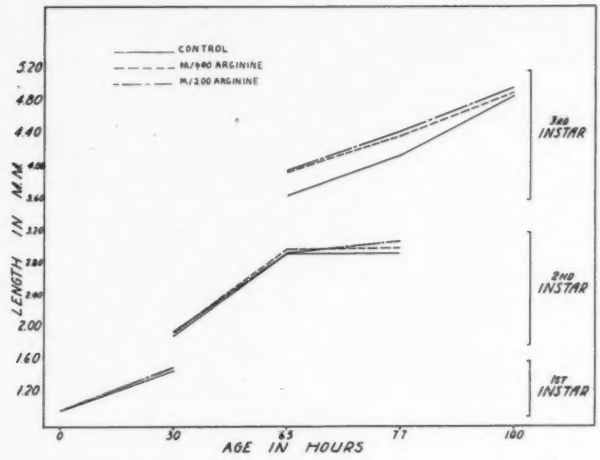

FIGURE 3

GROWTH IN LENGTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

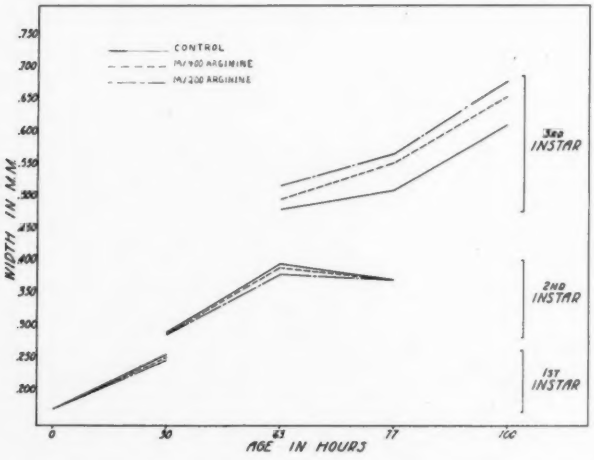

FIGURE 4

GROWTH IN WIDTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

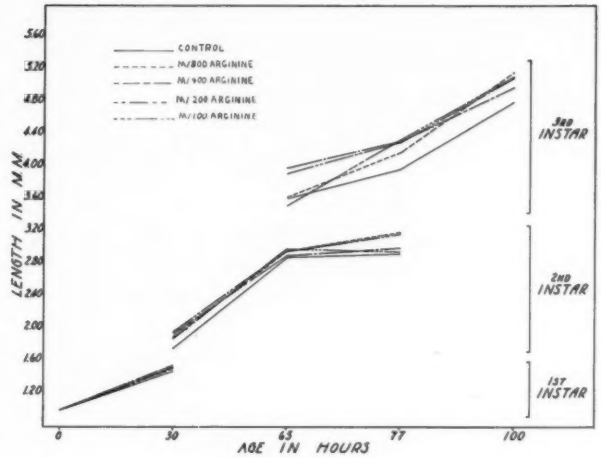

FIGURE 5

AVERAGE GROWTH IN LENGTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

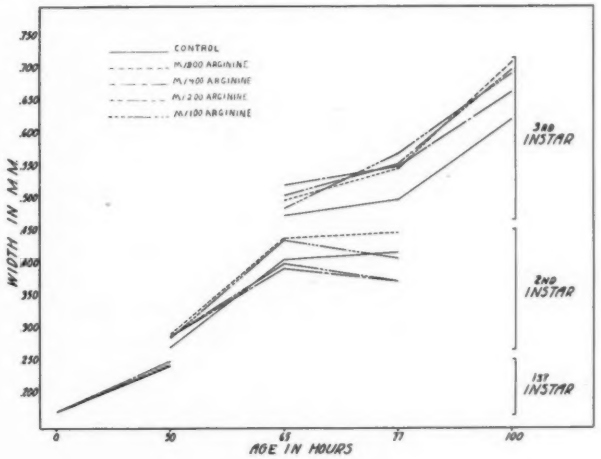

FIGURE 6

AVERAGE GROWTH IN WIDTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS FIVE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

tion of M/100 arginine is greater than that found in the yeast requirements (Meisenheimer, 1921); it therefore seems unlikely that a synthetic medium would contain a greater concentration.

It is evident that these four concentrations accelerated the rate of increase of the length and to a greater extent that of the width. The increase in size is evident in single experiments as in Tables 1 and 2 and in Figures 1, 2, 3, and 4, but is even more pronounced in averages of all the experiments (Table 3 and Figures 5 and 6). The increase of width in the tests is slight during the first and second instar but becomes comparatively great by the end of the third instar. In all cases the difference in width between test and control animals is more striking than that of length.

Regardless of the concentration of arginine in the test media, the percentages of difference in width and length of test over control animals are in the same range (Table *A*). This fact would seem

TABLE *A*

Concentration	Per cent of test over control	
	Width	Length
M/100	11	6
M/200	12	6
M/400	8	4
M/800	14	8

to indicate that here we are dealing with a specific acceleration effect rather than one concerned with nutrition or the amount of arginine ingested.

To test this hypothesis further two other concentrations of yeast were used; three grams per 100 cc. of culture medium and ten grams per 100 cc. When the amount of yeast was lowered, results, similar to those using five grams of yeast, were obtained. Typical measurements for a single experiment are shown in Table 4 and Figures 7 and 8. Here again both length and width were greater in test than in control (Table *B*).

TABLE B

Concentration	Per cent of test over control	
	Width	Length
M/200	5	8
M/800	9	7

TABLE 4

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH ARGININE (THREE GR. YEAST PER 100 CC.)

Age	I	Control			M/800			M/200		
		No.	Length	Width	No.	Length	Width	No.	Length	Width
34	1	28	1.51	.273	17	1.50	.251	11	1.47	.252
	2	9	1.80	.300	40	1.87	.293	36	1.89	.296
72	2	39	2.93	.405	28	2.99	.415	18	2.62	.400
	3	10	3.31	.458	26	3.51	.447	23	3.53	.453
96	2	11	2.72	.423	6	2.81	.400	4	2.78	.394
	3	28	3.78	.506	44	4.05	.527	42	4.01	.527
116	2	2	2.78	.375	5	2.52	.345	1	2.55	.375
	3	21	3.93	.507	15	4.23	.535	33	4.28	.545

I—instar.

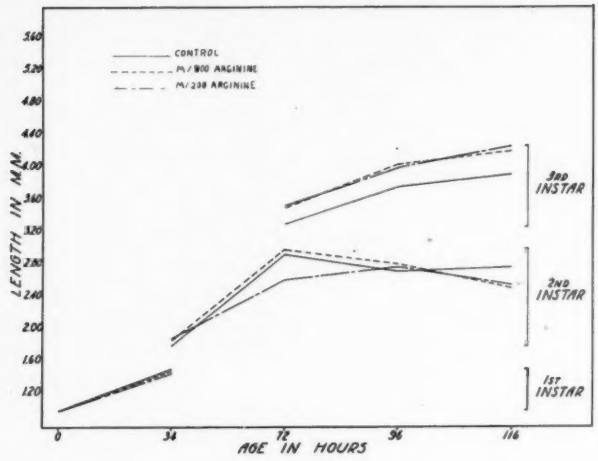

FIGURE 7

GROWTH IN LENGTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS THREE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

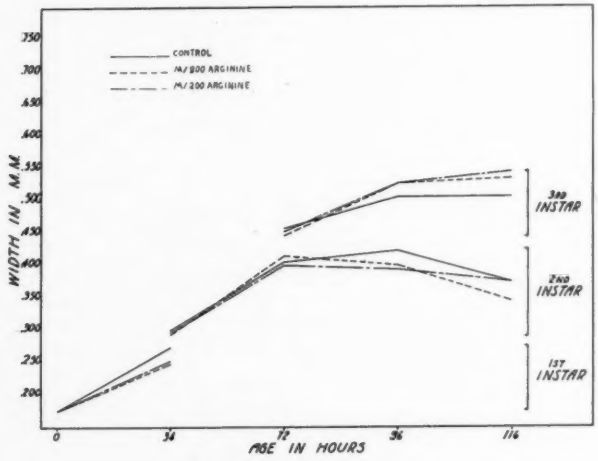

FIGURE 8

GROWTH IN WIDTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS THREE GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

Tests were also made using ten grams of yeast per 100 cc. of medium with a concentration of M/400 arginine. Results of a typical experiment are shown in Table 5 and Figures 9 and 10. Here the increase in width of the test over that of the control is 4 per cent and the increase in length is 2 per cent. When the optimum protein

TABLE 5

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH M/400 ARGININE (TEN GRAMS OF YEAST PER 100 CC.)

Age	Instar	Control No.	Control Length	Control Width	Test No.	Test Length	Test Width
24	1	31	1.45	.247	46	1.51	.258
	2	21	1.79	.286	14	1.81	.295
48	1	0			3	1.62	.300
	2	66	3.06	.470	53	3.11	.497
	3	11	3.68	.532	6	3.65	.537
72	2	1	2.33	.300	1	2.63	.375
	3	73	4.70	.655	61	4.87	.713
96	3	80	5.04	.723	82	5.16	.752

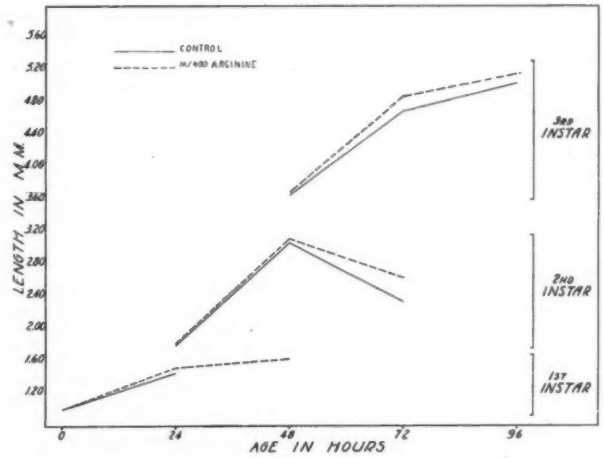

FIGURE 9

GROWTH IN LENGTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS TEN GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

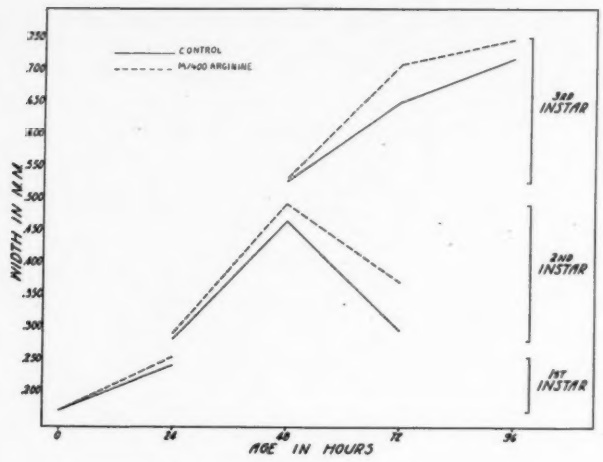

FIGURE 10

GROWTH IN WIDTH OF LARVAE OF DROSOPHILA TREATED WITH ARGININE PLUS TEN GRAMS OF YEAST PER 100 CC. OF CULTURE MEDIUM

requirement is more nearly met, there seems to be the same general trend but it is not so pronounced.

That lower concentrations of arginine are ineffective in modifying length or width of larvae is illustrated in Table 6 in which the results of three experiments using M/2,000 arginine are reported. The difference between tests and controls are small and are not in a definite direction. Similar results were obtained when M/10,000 arginine was used (Table 7). It seems therefore, safe to conclude that length of larvae are not modified by M/2,000 or lower concentrations of arginine. This amino acid may then, be tolerated by Drosophila larvae in all concentrations between M/100 and M/10,000 and concentrations between M/100 and M/300 are effective in producing an increased length and width.

When the rate of moulting and puparium formation as well as the duration of pupal periods were investigated, it was found that there was one more significant difference between test and control animals; an acceleration in the rate of moulting between the first and second instars occurred (Table 8). In every concentration from M/10,000

TABLE 6

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH M/2,000 ARGININE (FIVE GRAMS OF YEAST PER 100 CC.)

Age	Instar	Control No.	Control Length	Control Width	Test No.	Test Length	Test Width
			Experiment 1				
30	1	30	1.48	.240	27	1.51	.247
	2	13	1.86	.300	31	1.85	.295
65	2	18	2.95	.396	14	2.89	.407
	3	16	3.75	.482	22	3.41	.460
77	3	27	4.30	.525	19	3.90	.469
100	3	26	4.73	.608	35	4.71	.568
			Experiment 2				
30	1	22	1.52	.273	22	1.47	.256
	2	22	1.90	.300	25	1.87	.294
65	2	12	3.00	.437	8	2.95	.431
	3	9	3.58	.483	10	3.77	.533
77	3	21	4.12	.512	21	4.13	.561
100	3	23	5.17	.740	34	5.18	.748
			Experiment 3				
30	1	19	1.54	.261	15	1.54	.255
	2	27	1.92	.292	34	1.87	.302
65	2	10	2.94	.458	7	2.79	.429
	3	49	3.96	.525	36	4.04	.535
77	3	49	4.61	.608	44	4.63	.603
100	3	50	5.22	.725	42	5.18	.732

TABLE 7

AVERAGE LENGTH AND WIDTH IN MILLIMETERS OF LARVAE TREATED WITH M/10,000 ARGININE

Age	Instar	Control No.	Control Length	Control Width	M/10,000 No.	M/10,000 Length	M/10,000 Width
30	1	19	1.54	.261	17	1.51	.236
	2	27	1.92	.292	33	1.98	.302
65	2	10	2.94	.458	16	2.99	.437
	3	49	3.96	.525	40	3.90	.512
77	3	49	4.61	.608	40	4.77	.615
100	3	50	5.22	.725	35	5.21	.732

Age from median hour of egg hatching.

TABLE 8

THE INFLUENCE OF ARGININE ON MOLTING FROM FIRST TO SECOND INSTAR IN DROSOPHILA LARVAE AT 30 HOURS

Arginine Conc.	No.*	Total No. of individuals Control First instar	Control Second instar	Test First instar	Test Second instar	No. of experiments with more second instars Control	Test	Total % difference between No. second instars in test compared to control
M/100	4	92	84	53	140	0	4	+25%
M/200	4	110	143	57	180	0	4	+19%
M/400	3	91	109	79	111	1	2	+ 5%
M/800	3	79	47	62	91	1	2	+22%
M/2,000	4	82	95	74	132	0	4	+11%
M/10,000	1	19	27	7	33	0	1	+24

*Number of experiments.

TABLE 9

THE INFLUENCE OF ARGININE ON MOLTING FROM SECOND TO THIRD INSTAR IN DROSOPHILA LARVAE AT 65 HOURS

Arginine Conc.	No.*	Total No. of individuals Control Second instar	Control Third instar	Test Second instar	Test Third instar	No. of experiments with more third instars Control	Test	Total % difference between No. third instars in test compared to control
M/100	4	30	25	21	32	0	2	+ 7%
M/200	4	38	73	30	92	0	2	+11%
M/400	3	38	73	23	88	0	2	+14%
M/800	3	12	9	18	21	0	1	+11%
M/2,000	4	40	74	29	68	0	3	+ 6%
M/10,000	1	10	49	6	40	0	1	+ 4%

*Number of experiments.

TABLE 10
THE INFLUENCE OF ARGININE ON PUPATION

Arginine Conc.	No. of experiment	Total No. pupae counted Control	Test	Length of pupal stage Control	Test
M/100	4	242	186	3-4 days	3-4 days
M/200	4	110	129	4 days	4 days
M/400	4	331	348	3-4 days	3-4 days
M/800	3	106	139	3-4 days	3-4 days
M/2,000	4	258	240	3-4 days	3-4 days
M/10,000	1	86	79	4 days	4 days

to M/100 larvae moulted from first to second instars faster than did those in the control media. This acceleration was not maintained. for the percentage differences were lower at the second moult (Table 9) and were negligible at the time of pupation. Table 10 shows the duration of the pupal period which in no case is significantly different in test than in control.

Proline exerts a similar effect on first instar larvae (Wilson, 1941). The acceleration of moulting by proline might possibly be related to that produced by arginine for it has been found that for rats, ingested proline is a direct precursor for arginine (Stettin and Schoenheimer, 1943). These results do not agree with those of Hoffman and Gudernatsch (1931), with Hammett and Chatalbash (1935), nor with Miller and Reimann (1943) who found that differentiation was retarded by arginine. It may be pointed out that moulting between larval instars is not so clear cut an example of differentiation as that found in *Obelia* or as the metamorphosis of the tadpole into an adult. During the late third instar and pupal periods where great differentiation occurs in Drosophila, there was no acceleration. It is possible that arginine plays some part in the elaboration of the early moulting hormones but that the effect is lost as the larvae grow in preparation for pupation.

Certain structures might be concerned in decided width changes; the mass of the fat bodies, the thickness of the muscles of the body wall, or the size of the imaginal discs. In order to test the former, observations were made of cells in freshly excised fat bodies and camera lucida drawings made at a single level (Figure 11). It is difficult to obtain an accurate picture of conditions by this method but an impression was gained by several observers that at 77 hours

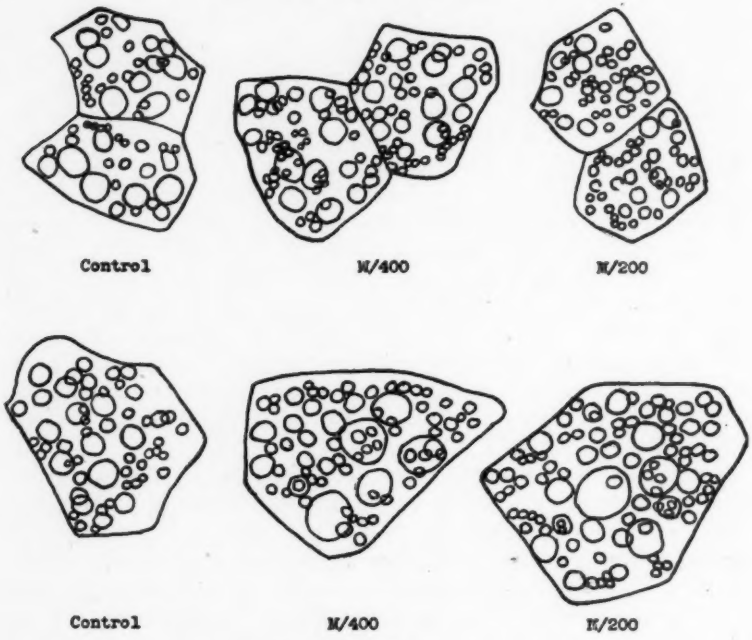

FIGURE 11

CELLS FROM THE FAT BODIES NEAR THE SALIVARY GLAND. UPPER ROW 77 HOURS FROM HATCHING; LOWER ROW, 100 HOURS FROM HATCHING

the test larvae had many more globules of fat in each cell than the control larvae. In addition these globules were smaller in size. Both of these observations indicate more centers of lipotropic activity.

At 100 hours the cells of the test larvae had larger vacuoles and the cells themselves seemed larger in size. Many measurements would be necessary in order for differences to be statistically significant because there is a great variation in cell size throughout the length of the fat body. The conclusion that development of the fat body is affected by arginine agrees with the observations of Lafon (1939) who calls this amino acid, essential for Drosophila. He believes that arginine is concerned in the formation of the fat body since larvae deprived of it were thin and failed to pupate. He made no measure-

ments and, as has been mentioned before, he did not supply the vitamin requirements.

Considering these results, it is obvious that some specific process which expresses itself by increase in width and slight increase in length is being accelerated. Since this greater mass is expressed regardless of available protein and since it seems independent of the concentration of arginine, one may assume that here we are dealing with a specific expression of a chemical reaction concerned in growth. Because of the augmented fat deposition in the fat body, one specific action of arginine is probably lipotropic.

If Drosophila formed fat from arginine directly as has been shown in some insects (Melampy and Maynard, 1937), more fat should be present in the fat bodies as the amount of arginine increased in the food. This however was not the case; once the amount of arginine which accelerated growth was present, additional amounts did not produce proportional increase in fat deposition. It would seem then that arginine itself is not converted into fat but that it in some way stimulates fat production.

Hammett (1942) clearly makes the distinction between substance increase which is constructive and that which is storage. He considers the former to be a result of growth processes and the latter not. According to this distinction, the effect of arginine on the fat body cannot be said to be that of furthering anabolic growth. It does, however, forward an increase in the reserve supply of fat available for future cytoplasmic activity.

In the larvae of Drosophila, most of the fat is stored in the fat body during the third instar, in preparation for the differentiation which occurs in the pupal stage. With more potential energy it would seem likely, therefore, that the fly would be larger at eclosion. Weights of the experimental adult flies did not, however, vary significantly from those of the control fly.

We did not investigate the imaginal discs which greatly increase in size by cell division during larval development. Brehme (1939) found that the thinner and shorter larvae of Minute had smaller imaginal discs, than the wold type larvae. It could be, therefore, that a part at least of the difference in size of the test larvae resulted either from an earlier development or a faster rate of mitosis of the imaginal discs or from both.

Hammett (1935) reports that in *Obelia*, arginine acts to accelerate the preparatory processes for new growth initiation rather than the processes concerned in proliferation as such. If this be the case in Drosophila, larger imaginal discs could result from earlier initiation of growth in the treated larvae. It is possible that the earlier moulting of the test animals from first to second instar is an expression of the participation of arginine in the initiation of new growth rather than its acceleration of differentiation as suggested above.

Some investigators (Mitchell and Roger, 1940; Gilroy, 1930; Shankman, 1943; and Kline, 1943) report that arginine is either essential for or stimulates cell multiplication. If it acts similarly in Drosophila, the structures in which mitosis normally occurs, such as the imaginal discs would be larger in the test than in the control and thus cause the greater width and length of the treated larvae. These investigators did not separate initiation of new growth from proliferation of cells, consequently, an exact interpretation of their results is impossible.

The possibility of phosphorylation of the ingested arginine and its subsequent storage in muscles of the body wall must not be overlooked. Just as arginine directly influenced creatine storage in muscles of vertebrates (Beard, 1941) it might also be concerned with storage of phosphoprotein in the muscles of the body wall of the larvae. We have however no evidence that structures other than the fat body are involved in the results of these experiments.

Summary

1. Arginine is tolerated by Drosophila larvae in concentrations from M/10,000 to M/100.

2. Concentrations of arginine from M/800 to M/100 accelerate the rate of increase in width and length of Drosophila larvae.

3. Concentrations of arginine from M/10,000 to M/100 accelerate the rate of moulting from first to second instars.

4. Arginine is ineffective in changing the rate of moulting between the second and third instars, the time of pupation, or the duration of the pupal period.

5. Arginine may have a lypotropic function in developing larvae.

6. Results are not inconsistent with the possibility that arginine participates in initiation of new growth.

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THE PRENATAL GROWTH OF THE CAT: XIV. THE WEIGHT OF THE SKELETON IN THE FETAL AND IN THE ADULT CAT

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The weight of the entire skeleton is known for man and for some of the domestic and laboratory animals, but so far as known there is but one report on the weight of the cat skeleton and this includes only four specimens (Welcker and Brandt, 1903). The weight of the human skeleton is given by Scammon (1933), Wilmer (1940), and Swanson and Iob (1940). The weight of the rat skeleton is probably best known through the earlier studies of Jackson and Lowrey (1912), Donaldson (1919), and all of the then known skeletal weights of the rat were summarized by Donaldson in 1924. The weight of the skeleton in the pig throughout the usual commercial slaughter weight (150-400 lbs.) has been studied by Loeffel. The skeletal weight in the fowl has been reported by Latimer (1924); Mitchell, Card, and Hamilton (1926); Latimer (1927); and for the turkey, Latimer and Rosenbaum (1926). The preparation of a vertebrate skeleton for the determination of its weight is a long and tedious process, but it seems that we should have more data on the mass of the entire skeleton in the various animal groups.

MATERIALS AND METHODS

This report is based on the skeletons of 229 fetal, 35 newborn, and 104 adult cats. The description of the materials and general methods are given in the earlier papers and need not be repeated here (Latimer and Aikman, 1931; and Latimer, 1936). The skeletons of the fetal and newborn cats had been preserved in formalin but the skeletons of the adult cats were weighed in the fresh condition. In other respects all of the specimens were treated in the same manner.

After the removal of the skin and its appendages and all of the viscera, the remaining musculature, skeleton and the contained central nervous system were weighed together, after all easily removable

fat, mesenteries and large vessels had been removed. The head and the two sets of extremities had been removed from the torso. Then each part was taken, one at a time and the musculature carefully cut from the skeleton. This was most easily and completely done by clipping it from the bones with a pair of scissors. Other methods were tried at first but this was found to be the most rapid and also yielded cleaner skeletons. All of the parts, other than that being worked on, were kept in a moist chamber and when the muscles had been removed from all of the skeleton, it and the contained central nervous system of the fetal and newborn cats were weighed, in a glass stoppered weighing bottle, on a chemical balance sensitive to 0.1 milligram. The adult cat skeletons were weighed on a laboratory balance sensitive to a tenth of a gram. The muscles were discarded, for the difference between the first and second weights gave the weight of the musculature, and it was felt that the weights of the parts were less subjected to drying by this method. Then the brain, spinal cord, and hypophysis were removed and weighed and the difference between this and the second weight gave the weight of the moist ligamentous skeleton.

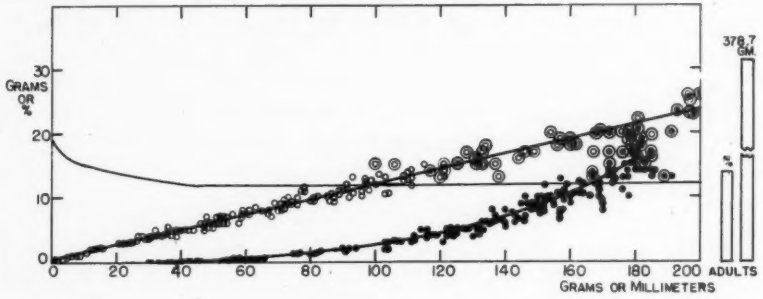

FIGURE 1

The straight line represents the growth of the skeleton plotted on body weight. The circles represent the individual weights of the fetal skeletons and the double circles, the skeletons of the newborn cats. The same skeletal weights plotted on body length are represented by the line concave superiorly and starting at 30 mm. of body weight. The dots represent the individual skeletal weights in the fetal cats and the dots enclosed by circles, the skeletal weights in the newborn cats. Empirical formulae for both curves are given in the text. The lighter line without any cases represents the percentage weight of the skeletons plotted on body weight. The abscissae represent either body weight in grams or body length in millimeters. The ordinates represent either weight in grams or percentage weights. The shorter column at the right represents the average percentage weight of the adult male skeletons and the taller broken column, the average weight in grams of the adult male skeletons.

The formulae for the growth of the ligamentous fetal skeleton on body length (Figure 1) was determined by the method of trial and error (Latimer, 1933). All of the rectilinear curves, for the fetal and for the adult cats, were fitted by the method of least squares (Snedecor, 1938). The formulae used in the determination of the statistical constants for the adult cats are those usually so employed and are given by Dunn (1929). All of the computations were carried to more decimal places than given in the tables and all were carefully checked, usually by two workers.

I wish to express my appreciation of the careful accurate work of Mr. Dale D. Lemon, who assisted with the routine work of preparing the tabular material for the adult cats.

Fetal and Newborn Skeletons

The growth of the fetal and newborn skeletons plotted against body weight and also against body length is shown in Figure 1. The upper rectilinear curve with the individual skeletal weights shown as circles for the fetal cats and as double circles for the newborn cats, represents the increase in skeletal weight when plotted on body weight and with both weights measured in grams. There is no evident change in the rate of growth of the newborn and all of these fetal and newborn cats are fitted by the one formula. The empirical formula from which this curve was drawn is $Y = 0.1158X + 0.34$, from 5 to 200 grams of body weight. The average percentage deviation of the calculated from the observed values for each 10 grams increase in body weight is 2.16 per cent, which shows that this curve fits the cases very well.

The curve beginning at 30 millimeters of body length and with the weights of the individual fetal skeletons shown as dots and the newborn skeletons shown as dots enclosed by a circle, was drawn from the empirical formula: $Y = \frac{(0.1X)^3}{377} - 0.002X$. This formula may be used between 40 and 185 millimeters of body length. This includes most of the newborn. There are a few newborn cats of a greater body length but the curve comes very close to these and so when the body length is used, as well as for the body weight, one curve will locate all of the skeletal weights for both the fetal and the newborn cats. This curve does not fit the cases quite as well as the

curve for body weight, for in this case the average percentage deviation is 5.88 per cent, but this is a good fit.

The lighter line in this figure, or the line without any cases, represents the changes in the percentage weight of the skeleton. The skeletons expressed as percentages of the body weight were plotted on body weight and from these cases this smoothed curve was drawn. This shows that the fetal skeleton starts at about 18 per cent of the body weight and after decreasing rather rapidly from 0.3 grams to 45 grams of body weight, continues as a straight horizontal line. The average percentage weight of the skeleton in all of the fetal cats with a body weight of 45 grams or above and all of the newborn cats is 11.99 per cent. There was no significant change in the newborn cats. The average percentage weight of the skeleton in the newborn cats is 11.70 per cent or a difference of only 0.29 per cent from that of the average of the entire group above 45 grams of body weight. This again shows that there is no significant difference in the rate of growth for the weight in grams of the skeletons or in the percentage weight of the skeletons of the newborn cats compared to the fetal cat skeletons.

The shorter column at the right represents the average percentage weight of the skeleton in the adult males. The percentage weight in the females is slightly less, as shown in Table 1. This means that the average percentage weight of the skeleton changes less than two per cent of the body weight in the fetal cats above about 45 grams of body weight, in the newborn cats and also in the adults. We have no data on the possible changes during postnatal growth. The taller, broken column represents the average weight in grams of the adult male skeletons.

Adult Skeletons

The average, the coefficient of variation, and the significant difference for the male and the female adult cats are given in Table 1 for both the weight of the skeleton in grams and for their weights as percentages of the body weight. The male skeleton is heavier than the female and the last column shows that it is significantly heavier, for the difference between the weights is over six times the probable error of the difference. Also the weights of the male skeletons are more variable, nearly 60 per cent more variable than the female skeletons.

TABLE 1

WEIGHTS AND PERCENTAGE WEIGHTS OF THE ADULT SKELETONS

	Males		Females		
	Average weight	Coefficient of variation	Average weight	Coefficient of variation	Significant difference
Skeleton in grams	378.69±7.70	21.30±1.50	325.47±4.23	13.33±0.93	6.06
Skeleton as per cent of body weight	13.84±0.20	15.44±1.06	13.38±0.21	16.26±1.15	1.59

The percentage weights are slightly, but not significantly greater in the males. These percentage weights are a little over five per cent more variable in the females.

The total increase in skeletal weight during the fetal period is 129 times, and during this period the body weight is increasing 195 times. In the postnatal period the body weight increases about 20 times (22.3 for the males and 19.9 times for the females), and the male skeletons increase 26.0 times and the females, 22.3 times, or the skeletal weight increases a little less than the body weight in the fetal period and a little more than the body weight in the postnatal period.

In general the skeletons of these cats form about 18 per cent of the body weight in the smallest fetuses, this rapidly decreases and remains at about 12 per cent up to and including the cats at the time of birth and then increases slightly to between 13 and 14 per cent in the adults. Welcker and Brandt (1903) find an average of 11.53 per cent for their four adult specimens. The human skeleton seems to be a little heavier, for Wilmar (1940) reports the human skeleton at the sixth fetal month as forming 22 per cent of the body weight, 17.69 at birth and 17.60 in the adult. Scammon (1933) gives the skeletal weight in man from 15 to 20 per cent of the body weight at birth and remaining at about that figure to maturity. Loeffel in some unpublished data on the percentage weights of the skeletons in pigs finds that the skeleton forms 13 per cent in pigs of 150 lbs. of body weight and that this percentage decreases with uniform velocity to 8 per cent in pigs of 400 lbs. of body weight. Both Jackson and Lowrey (1912) and Donaldson (1924) find a decrease in the percentage weight of the rat skeleton in postnatal life. The chicken skeleton increases in percentage weight in postnatal life (Latimer, 1927). There seems to be a slight sex difference in the percentage weight of the cat skeleton, and a significant sex difference in the chicken skeleton (Latimer, 1927), with the males heavier in both cases.

Empirical formulae have been developed by the method of least squares, for the weights of the skeletons on body weight or on body length for the two sexes separately. While the average weights and average percentage weights given above are good for cats of average weight, these formulae will predict the skeletal weight more accurately for cats other than those of average body weight.

The formulae for the male cats are as follows: for cats with body weights ranging from 1,700 to 4,500 grams, $Y = 0.085X + 143.1$ and for cats with a body length ranging from 450 to 600 mm., $Y = 1.57X - 428.65$. In these formulae Y represents the skeletal weight in grams and X represents either the body weight in grams or the body length in millimeters. There is an average percentage deviation of 2.52 per cent for the first curve, on body weight, and 5.07 per cent for the formula based on body length. These curves are all straight lines and with a moderate scatter in the distribution of the individual specimens, and to save space none of them are reproduced.

The percentages of the skeleton were plotted on body weight and a smoothed curve drawn through the averages for each 500 grams of body weight and this curve started at 18 per cent in the smallest male cats and decreased to 12 per cent in the largest cats or practically the same percentages found in the fetal cats. The curve decreased regularly to about 3,500 grams of body weight and from this point on the percentages averaged 12.16 per cent.

The formulae for the female cats from 1,500 to 3,500 grams of body weight are as follows: $Y = 0.052X + 195.18$, and for cats from 450 to 570 mm. in body length, $Y = 1.1X - 240.04$. In both of these formulae, Y represents the skeletal weight in grams, and X represents either the body weight in grams or the body length in millimeters. The average percentage deviation for the first formula, based on body weight is 1.05 per cent and for the second, 1.88 per cent. This would indicate that the curves for the females were a little better fitted than those for the males, although all of them are well fitted.

The percentage curve for the female skeletons is a straight line decreasing from 16 per cent in the smallest cats to about 10 per cent in the largest specimens. The two curves for the percentage weight of the skeleton plotted on body weight in the adult male and female cats very closely resemble the curve for the percentage weight of the skeleton in the pig from 150 lbs. to 400 lbs. of body weight (Loeffel), except that the skeleton in the pig forms a constantly smaller percentage of the body weight. Thus we see that in the adult cats of both sexes, the skeleton forms a larger percentage of the total weight in the smaller cats. Doubtless a part of the smaller percentage value in the larger cats is due to the accumulation of fat, but the decrease in the earlier part of the range is due without doubt to the

relative increase in the percentage of the musculature, as will be shown in a later paper.

Correlations between the weight of the skeleton and some of the organs of the males and the females are shown in Table 2. All of the

TABLE 2

CORRELATIONS WITH THE WEIGHTS OF THE SKELETONS

	Males	Females
Body weight	+0.783±0.037	+0.550±0.063
Body length	+0.758±0.041	+0.554±0.057
Integument	+0.812±0.032	+0.590±0.063
Musculature	+0.662±0.054	+0.410±0.081
Heart	+0.722±0.046	+0.587±0.064
Spleen	+0.395±0.080	+0.273±0.090
Brain	+0.516±0.070	+0.231±0.092
Spinal cord	+0.866±0.024	+0.684±0.052
Eyeballs	+0.755±0.041	+0.560±0.067
Digestive tube weight	+0.755±0.041	+0.566±0.066
Digestive tube length	+0.578±0.063	+0.292±0.089
Liver	+0.470±0.074	+0.395±0.082
Pancreas	+0.415±0.080	+0.352±0.085
Lungs (clear lungs only)	+0.825±0.036	+0.521±0.056
Kidneys (both)	+0.715±0.046	+0.594±0.063
Bladder, urinary	+0.816±0.032	+0.365±0.084
Gonads	+0.607±0.060	+0.401±0.083
Uterus	———	+0.276±0.095
Thyroid	+0.564±0.065	+0.279±0.090
Suprarenals	+0.669±0.053	+0.488±0.074
Hypophysis	+0.231±0.091	+0.458±0.078

correlations are positive, but four of the male correlations and 12 of the correlations in the females are below 0.5 and are not significant. The 20 correlations with the male skeletons average + 0.646 and the 21 female correlations average + 0.449. The highest correlation in both sexes is with the spinal cord. This correlation in the males is +0.866 and in the females +0.684. The correlation with the spinal cord is the only correlation above 0.6 in the females, but there are 13 correlations of 0.6 or above in the males. The spinal cord and the integument are the only two organs found in the highest five correlations in both sexes. The brain, the spleen, and the pancreas are the three correlations which are found in both sexes in the lowest five correlations. Many organs have a high correlation with the skeleton in one sex and a low correlation in the other, for example, the bladder is third from the highest in the male correlations and seventh from the lowest in the females. In general, the male skeletons are better correlated with the other organs than are the female skeletal weights.

Summary

Empirical formulae are given for the determination of the weight of the ligamentous skeleton from either the body weight or the body length. In the fetal and newborn cats, the skeletal weight increases as an arithmetical function of the body weight and as a geometrical function of the body length. All of the curves for the adult cats are straight line curves.

The skeleton decreases from its maximum of 18 per cent of the body weight in the smallest fetal cats to about 12 per cent in the newborn, and to between 13 and 14 per cent in the adults. The percentages in the adult females are a little lower than in the males.

The weights of the adult skeletons are significantly greater in the males and they form larger, but not significantly larger percentages of the body weight in the males. The skeletons are more variable in the males but the percentage weights are more variable in the females.

The correlations with the body weight, body length, and the weights of many organs average higher in the adult males. All of the correlations are positive, but four of the correlations in the males, and 12 in the females, are not significant.

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CHARACTERISTICS OF THE DAILY WEIGHTS OF SEXUALLY MATURE RATS ON A CONSTANT DIET

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A. Introduction

Biological assays of vitamins, toxicity, dietary efficiency, and the like are often based upon changes in the weight of laboratory animals. The animal most widely used for such purposes is the rat. These biological assays as described by Coward ('32) and Bliss and Cattell ('43) are based on the weight curves, although this fact is often not explicitly recognized. The intrinsic weight curves of the different rats are assumed, for instance, to remain the same throughout an experiment. Closer scrutiny of the weight characteristics of rats may lead to a decided change of technic in biological assay. Instead of a "large" number of supposedly uniform rats, it may be advantageous to use a smaller number on which more extensive data are collected. In any event, rats do behave as individuals with respect to their weight curves.

After the daily weights of 14 genetically similar, sexually mature, female rats in this laboratory had been examined for 94 consecutive days, it was found that the daily weights of each could be expressed as a polynomial trend plus a periodic part which in almost every case is the sum of two sine curves plus a nonperiodic erratic part. This last part is not exactly random, for the successive observations still tend to exhibit a slight correlation.

These findings agree with the results of other investigations. Wishart ('39) has used orthogonal polynomials to represent change in the weight of pigs with time. Maymone and Sircana ('30) showed that the daily weights of dairy cows for a period of 75 days could be fairly well represented by a straight-line (first degree polynomial) trend plus a sine curve. According to data of Baker and Guilbert ('42) on 69 consecutive daily weighings of seven heifers and one steer, all yearlings, the daily weights consisted of a trend plus an os-

cillatory part that was roughly cyclic. The results of Baker and Mead ('42) with dairy heifers confirmed these findings and indicated, furthermore, that for younger animals the periods may be longer and more distinct.

Knudsen ('42) advocates the use of fifth-degree polynomials to represent the complete growth of rats. Using weekly weights, she notes that the successive residuals from the polynomials show fairly high correlations, indicating cycles or periodicities; the result, she says, was to be expected. Furthermore, she questions the use of the ordinary tests of significance for nonindependent residuals.

Knudsen relied on weekly weights and did not suggest definite periods. By considering daily weights, however, the present writers have been able to isolate periodicities that account for nearly all the non-randomness of residuals from polynomial trends.

B. Data

The data used in this study were gathered in connection with an experiment on K-deficient diets. These 14 female rats, the control animals, were fed 10 grams of a normal diet each day. They were weighed daily. The time considered is July 28 to October 29, 1943. The rats were kept in a good laboratory under as uniform conditions as possible without air-conditioning. The 94-day period started five days after the beginning of the experiment and ended when the amount of feed given was changed to correspond to the amounts taken by rats on the K-deficient diet in the same groups. Temperature of the rat colony seems not to have had any consistent bearing on the changes in weight.

C. Treatment of Data

Third-degree orthogonal polynomials were fitted to the daily weights of each rat as indicated by Anderson and Houseman ('42). Third-degree polynomials seemed adequate for the portion of the growth considered. Their constants are presented in Table 1. The A'_0's are the average weights over the 94-day period, the A'_1's give the average gain in weight per day, the A'_2's are proportional to the change in gain per day, and so forth.

The averages of the trend coefficients in Table 1 give the trend coefficients for the average of the group of 14 rats. The average 0.0556759 of the A'_1 coefficients shows that the average linear trend

TABLE 1

CONSTANTS FOR THIRD DEGREE ORTHOGONAL POLYNOMIALS FIT TO THE 94 DAILY WEIGHTS IN GRAMS OF 14 FEMALE CONTROL RATS WITH THE INDICATED AGES AT THE BEGINNING OF THE PERIOD

Rat. No.	Age (days)	A_0' (Mean)	A_1'	A_2'	A_3'
B 4496	89	162.28	.1193946	.0057326	—.000000481
B 4482	90	163.01	.0827836	.0125360	.000070239
B 4437	90	170.08	.1446158	.0150752	—.000023946
G 4474	91	177.46	.0248998	.0043502	.000052831
B 4467	91	161.73	.0296030	.0058663	.000030603
G 4454	96	163.38	.0093010	.0096883	.000000193
G 4404	98	148.36	.0311166	.0038057	.000044631
G 4442	97	165.15	.0438898	.0016456	.000067728
G 4416	97	151.90	.0094354	.0111460	—.000134512
G 4414	97	154.02	.0650977	.0117616	—.000005226
W 4403	98	139.13	.1132464	.0077857	.000061904
G 4385	103	157.05	—.0060290	.0125142	.000006643
W 4384	103	164.27	.0492540	.0078880	.000013038
G 4373	105	157.45	.0628544	.0007047	.000023210
Average	96	159.66	.0556759	.0078932	.000014775

of the weights of the rats amounts to 5.2 grams over the 94-day period. The individual A'_1's show, however, that the rats did not make average daily gains in weight and that one rat had a negative average daily gain. The third-degree trends, if plotted, are seen to differ decidedly from rat to rat, that is, each rat follows an individual trend, not obviously associated with initial or mean weight.

The individualized trend of a rat's weight is confounded in observation with the trend produced by the treatment, especially in vitamin and toxicity trials, so that accuracy of such trials may be greatly reduced for this reason. If extensive enough data were available on each rat, a trend might be calculated and then extrapolated through the experimental period; hence the change in trend due to the treatment might be obtained free of the intrinsic trend for the rat. This method would be especially important for studying the variation in individual responses to a treatment. It has been suggested in principle by Baker ('43). Another usual method is to have two groups of rats, one treated and the other control. It is then erroneously assumed that the two groups have the same intrinsic average trend and that the difference in trend results from treatment.

Let us consider the gain in weight from one time to another, selecting the time interval so that periodicities in the weights are eliminated. We can do this for 12 rats by choosing a period of 54 days. The residual mean square for these rat weights with the periodicities removed is 4.0. If there was no difference in trend for the 12 rats, the variance of a gain in weight would be 2 times 4.0 = 8.0; and the expected gain would be the same for each rat. Since there is a difference in trend for each, let us see what effect this difference has if we take the gain for the first 54 days for these 12 rats. Let $m_i (i = 1, 2, —, 12)$ denote the expected gains in weights as computed from the trends. Then we have to combine 12 random variables with expected values m_i, each with a variance of $2\sigma_i^2$ where σ_i^2 is the residual variance for the ith rat. Let σ_T^2 be the total variance of gains in weight at the end of the 54-day period, and let σ_m^2 be the variance due to differences in the m_i. Then following Yule and Kendall('37), we have

$$\sigma_T^2 = \frac{2}{12} \sum_{i=1}^{12} \sigma_i^2 + \sigma_m^2.$$

In the present case we compute $\sigma_m^2 = 40.2$, $\frac{2}{12} \sum \sigma_i^2 = 8.0$; hence $\sigma_T^2 = 48.4$. Thus the variance is increased sixfold because of differences in trend; and 6 times as many rats are therefore required to insure the same accuracy in a gain-of-weight determination with these differences in trend for a 54-day period as for a similar experiment when the trends are the same.

Residuals computed from the polynomial values for each rat, were plotted. They showed pronounced periodicities. Following Davis's ('41) procedure, sine curves were fitted to the residuals from the trend polynomials; the results appear in Table 2. It should be noted that only part of the observed data are available for determining the periodic components for long periods.

As Table 2 shows, 12 of the 14 rats have periodicities in their daily weights that can be represented well by a sine curve of period 27 days or by the combination of a sine curve of period 27 days and a sine curve of period 54 days. The other two rats, G 4416 and W 4403, have periodicities that can be represented well by the com-

TABLE 2

Constants for the representation of the periodic part of the daily weights in grams of 14 rats in the form $R_1 \sin\left(\frac{2\pi t}{T_1} + \alpha_1\right) + R_2 \sin\left(\frac{2\pi t}{T_2} + \alpha_2\right)$ where t is time in days; R_1 and R_2 the amplitudes; T_1 and T_2 the periods; and, α_1 and α_2 angles in radians which adjust the curves to the proper starting point.

Rat. No.	R_1	T_1	α_1	R_2	T_2	α_2
B 4496	2.0735	27	5.1310	0.9874	54	5.3332
G 4482	1.3229	27	4.7696	0.9096	54	5.3262
B 4437	0.4749	27	2.8457	1.3510	54	2.3591
G 4474	2.3629	27	5.0010	1.0415	54	5.3640
B 4467	1.4344	27	4.6775	1.8931	54	5.4002
G 4454	2.7317	27	4.3328	3.1750	54	5.1677
G 4404	2.8460	27	4.9902	1.2141	54	0.7447
G 4442	0.8545	27	4.8399	2.1904	54	5.5996
G 4416	2.2240	36	3.4214	4.0185	72	5.5047
G 4414	1.4799	27	4.2626	1.7889	54	5.5367
W 4403	1.6881	36	1.6714	4.1016	72	2.1132
G 4385	1.4254	27	4.4434	2.7103	54	5.9719
W 4384	1.2505	27	4.6251	1.5568	54	5.4658
G 4373	1.9447	27	0.0670	———	—	———

bination of a sine curve of period 36 days and a sine curve with a period of 72 days.

The indicated periods are long, considered from the standpoint of the life span and developmental period of the rat. If rats of different ages were considered, the periods might be of different lengths. If so, we could not expect to extrapolate the curves with the periods found much beyond the observed range unless further investigations were made.

The residuals of the polynomial residuals from the sine representations of Table 2 were computed and plotted. They were erratic and showed no further evidences of periodicities. Some of the daily weight deviations from trend were very large. One rat, G 4404, refused some feed for a day or so; this lack of appetite markedly influenced weight. Other rats may have had temporary upsets which did not change the amount of food eaten but which nevertheless affected the daily weights.

If periods are taken so that the periodicities in the weights are not eliminated, then the variance of a gain in weight is still further increased; hence the number of rats required to give an accuracy equal to that obtained in the case where the trends are equal and no periodicities exist may well be increased tenfold.

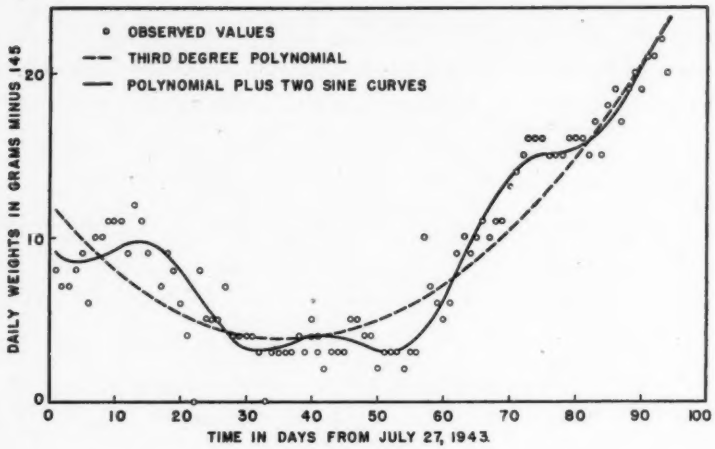

FIGURE 1

OBSERVED AND "EXPECTED" DAILY WEIGHTS IN GRAMS MINUS 145 OF RAT G 4414

The expected weights, y, are given by $y = 9.021 + 0.050977\ \xi_1^1 + 0.0117616\ \xi_2^1 - 0.000005226\ \xi_3^1 + 1.4799 \sin\left(\frac{2\pi t}{27} + 4.2626\right) + 1.7889 \sin\left(\frac{2\pi t}{54} + 5.5367\right)$, where t is time and ξ_1^1, ξ_2^1, ξ_3^1 are orthogonal polynomials in t.

Figure 1 shows the observed weights, third-degree polynomial trend, and combined polynomial trend and sine curve periodicities for one rat, G 4414. The picture for other rats is very similar, but of course there are individual variations: For some the amplitudes of the sine components are much greater; for others smaller. The two components are shifted different amounts with respect to each other for the different rats. That is, α_1 and α_2 do not bear a constant relation to each other for all rats. For rat G 4414 a few of the weights differ widely from proceeding or succeeding weights. The phenomenon is characteristic of many of the other rats also. All the difference due to periodicities can be eliminated in 12 cases, however, by considering a period of 54 days and in two cases by considering a period of 72 days. These facts are rather important in planning experiments.

The variations in the daily weights of these 14 female rats are analyzed in Table 3 into parts due to polynomial trend, periodic component, and residual. Polynomial trend and periodic components

TABLE 3

ANALYSIS OF VARIANCES OF DAILY RAT WEIGHTS IN GRAMS

Rat. No.	Total sum of squares	** *DF*	Sum of sq. of dev. from poly. trend	*DF*	Sum of sq. due to poly. trend	DF	Sum of sq. of deviations from sine curves	*DF*	Sum of sq. due to constants of sine curves	*DF*	Residual mean square
B 4496	4,872.00	93	591.79	90	4,280.21	3	385.55	86	206.24	4	4.48
G 4482	4,172.10	93	358.12	90	3,813.98	3	286.20	86	71.92	4	3.33
B 4437	8,493.58	93	351.78	90	8,146.80	3	289.13	86	62.65	4	3.36
G 4474	1,045.96	93	505.71	90	540.25	3					
B 4467	1,038.44	93	386.84	90	651.60	3	288.28	86	217.43	4	3.35
G 4454	4,120.16	93	769.56	90	3,350.60	3	178.13	86	208.71	4	2.07
							226.88	86	542.68	4	2.64
G 4404*	1,720.73	93	1,212.98	90	507.75	3					
G 4442	1,753.77	93	843.62	90	910.15	3	679.60	86	533.38	4	7.90
G 4416	1,894.61	93	1,397.95	90	496.66	3	663.42	86	180.20	4	7.71
							506.08	86	891.87	4	5.88
G 4414	3,024.43	93	435.76	90	2,588.67	3					
W 4403	4,717.63	93	1,345.13	90	3,372.50	3	229.89	86	205.87	4	2.67
G 4385	2,321.10	93	712.53	90	1,608.57	3	390.61	86	954.52	4	4.54
							423.83	86	288.70	4	4.93
W 4384	1,760.29	93	444.00	90	1,316.29	3					
G 4373	1,458.93	93	319.62	90	1,139.31	3	276.98	86	167.02	4	3.22
							176.04	88	143.58	2	2.00
Total	42,393.73	1,302	9,675.89	1,260	32,723.34	42	5,000.62	1,206	4,674.77	54	4.15

*Refused some feed.

**Degrees of freedom.

always result in a highly significant reduction of the sum of squares. The ordinary F-tests are used as the basis for this statement, since the final residuals are approximately normal and not far from random. If the residual mean squares are tested for homogeneity as indicated by Roessler ('43), the variances are found to be almost surely non-homogenous. This means that some rats, though genetically very similar, are naturally more variable in their daily weights than others even after the trend and the periodic components are removed.

D. Summary

Apparently the daily weights of female rats, 89 to 105 days old, fed on a constant normal ration under standard laboratory management, can be expressed as a trend that can be represented by a polynomial, plus a periodic component that can be represented by one sine curve or by the combination of two sine curves, plus a part that is nearly but not quite random. This residual mean square or variance when the weights are in grams varies from 2 to nearly 8. According to the present data, one can eliminate perhaps nine-tenths of the variance of gain in weight of individual rats on a constant diet by considering the trends and periodicities in rat weights.

The uncorrected gains in these 14 rats over the entire period had a variance of 65. By fitting trends and considering periodicities we can break this variance into two parts: one due to residual error, which is $2 \times 4.2 = 8.4$, and the other due to differences in the expected values in the gains of weights. Thus the variances of the corrected weights would be 56.6, which is 87 per cent of the variance of the uncorrected gains. Evidently, therefore, the variation between these rats is still important even though they are genetically closely similar.

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Growth, 1944, **8**, 169-171.

CHARLES B. DAVENPORT

(An Editor of Growth)

Charles B. Davenport was a great man. Great as a scientest. Great as a person.

He was truly a scientist. He thought more of what he could give to science than what science could give to him. And he gave bountifully to science.

It was through him that American workers were stimulated to an interest in human growth; and to an interest in eugenics. And it was he who not only demonstrated to American workers the utility and need for statistical analysis of biological data; but also gave to them a handbook of statistical methodology which has yet to be equalled in clarity and conciseness. This little volume is a classic and will remain a classic unsmothered by the prolific and confused verbiage of tomes from later pens.

It, is, however, of Charles Davenport the person that I would write. And of his patient helpfulness to me, a younger and awkward worker.

My first personal meeting with him was when I was somewhat unwisely invited to tell the Cold Spring Harbor group of my thyroid studies. As was the pleasant custom of the 1920's the guest speaker was dined and also wined at Oyster Harbor before the evening meeting. It happened that I was too well wined. With the result that my discourse was given in a hilarity-producing alcoholic haze. The hilarity coming not only from my inability to enunciate my key words thyroparathyroidectomized and parathyroidectomized with any syllabic continuity; but also from my obvious sheepish chagrin at this inability. I shall never forget the kindly twinkle, and the friendly squeeze which Dr. Davenport gave me after this painful exhibition of incoördination as he said to me: "Young man! Next time you are invited to speak, be careful what you drink!"

This understanding, interest, and helpfulness is to me the mark of his character as a person. For it was evinced on many a later less embarrassing occasion.

Thus—when I was struggling to find a way to report the

results of my *Obelia* experiments he laid aside his work and duties at Cold Spring Harbor and came here to North Truro, to see what could be done with the data. For two days he studied and analyzed the intricate records. And from that study he resolved my uncertainty by concluding that to report the results in terms of consistency in direction of deviation would not only be valid, but also intelligible, at least to those who are willing to sit instead of run when they read.

That this great Director of a great Institution should have been willing to make the tiresome and tedious trip from Long Island to the tip of Cape Cod just to help a puzzled young worker out of his difficulty, is evidence enough that Charles Davenport was not only a great scientist, but also a great person.

Mrs. Davenport, that fine lady and companion, came with him and they honored us by staying at our home. The affectionate regard which Mrs. Davenport had for her husband was shown in her respect for him and the respect which she demanded that others show. I still remember blushingly how she gently chided me when I started to interrupt Dr. Davenport as he was making a point in a discussion taking place while we were at dinner, as she said: "Mr. Davenport is talking, Mr. Hammett." And I still remember the affectionate way in which she looked at him, and the little smiling wrinkles around his eyes as he glanced at me and then looked silently at her. It was gemütlich.

The companionship between these two must have been very real, because each time Dr. Davenport attended the meetings of the Editors of GROWTH here in Provincetown, he brought Mrs. Davenport with him. And as it became increasingly difficult for her to get around he became increasingly solicitous that their accommodations should entail as little difficulty as possible. And finally when she could no longer come, he himself would not come.

I think the extent of his participation and help in the beginning of GROWTH has been realized by but few of those who are now Editors or readers of our Journal. When I

became convinced that those of us who are trying to solve one or another of the multifarious problems of growth needed a journal in which we could present our data and ideas unrestrictedly in order that a beginning integration might be had, I wrote to all those who had recorded themselves in *American Men of Science* as working in that general field. Of the many who approved of the idea and the idea that institutions should pay for publication as well as for the conduct of research, Dr. Davenport was one of the few who offered to help get such a journal started. And as before, he laid aside his duties at Cold Spring Harbor, and came here, not once, but twice and his wisdom at these preliminary organizing conferences were deciding factors in bringing about the establishment of our Journal. Without his self-sacrificing and unselfish help we might never have come to fruition. And his backing was a valuable help in getting the other Editors to approve the idea of an annual symposium on growth.

It can truly be said: What a man! For here we have one who could afford and deserved to rest on his well earned laurels, who might have been expected without criticism, to be "sot in his ways," because of his 70 odd years, not only interested in, but also actively participating in the promulgation of a project which to say the least was a break with tradition.

Charles Benedict Davenport was not only a great scientist: he was a great person.

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BOOK REVIEW

Growth, 1944, **8**, 173.

(F. C. Bawden. Plant Viruses and Virus Diseases. Waltham, Mass.: Chronica Botanico. Pp. 294.)

REVIEWED BY FREDERICK S. HAMMETT

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With the invasion of the Netherlands the type of the First Edition of this eminently valuable compilation was lost. Despite the war, work on viruses and virus diseases has made considerable progress, and the need to set up new type was made the occasion for bringing the text up to date and adding new illustrations. Though an attempt has been made to include all recent work the difficulties of getting literature means that some has inevitably been missed. Nonetheless the bibliographies appended to each chapter seem to the reviewer to cover the ground better than could be expected under the circumstances. The increasing recognition of the potential importance of the viruses or virus-possible agents in pathological growth as exemplified in the outstanding discoveries of Bittner make this book of Bawden's an almost necessary member of the library family of all students of growth in its integrating aspects. While it is true that the subject-matter is weighted to the plant viruses the fundamental principles of transmission, mutation, immunity, serological reactions, methods of purification, properties, together with the data on inactivitation and size of virus particles here given can well be guides to extension of exploration into similar agents affecting animal tissue and growth. And the discussion on origin and multiplication of viruses should make all biologists furiously to think.